ading

HEMISTRY AND INDUSTRY
OF STARCH

SECOND EDITION, REVISED AND ENLARGED

Edited by

RALPH W. KERR, PH.D.

Corn Products Refining Company, Argo, Ill.

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, * MYSORE. *
4 MAR 1951
LIBRARY

1950
ACADEMIC PRESS INC., PUBLISHERS
New York, N. Y.

1409 ✓

COPYRIGHT 1950, BY
ACADEMIC PRESS INC.
125 EAST 23RD STREET
NEW YORK 10, N. Y.

El,58768

N50;1

NO PART OF THIS BOOK MAY BE REPRODUCED IN ANY FORM, PHOT
MICROFILM, OR ANY OTHER MEANS, WITHOUT WRITTEN PERMIS
FROM THE PUBLISHERS

PRI ES OF AMERICA

CFTRI-MYSORE
1409
Chemistry and in.

CO-AUTHORS

George V. Caesar, *The Huron Milling Company, Harbor Beach, Michigan*

Leo M. Christensen, *Consulting Chemurgist, Lincoln, Nebraska*

Ed. F. Degering, *Armour Research Foundation, Chicago, Illinois*

Dexter French, *Iowa State College, Ames, Iowa*

Alexander Frieden, *Director of Research, Pabst Brewing Company, Milwaukee, Wisconsin*

Eric Kneen, *Director of Research, Kurth Malting Company, Milwaukee, Wisconsin*

J. M. Newton, *Director, Technical Sales Service, Clinton Industries, Clinton, Iowa*

George Peckham, Jr., *Research Supervisor, Clinton Industries, Clinton, Iowa*

Garrish M. Severson, *University of Nebraska. Lincoln, Nebraska*

PREFACE TO SECOND EDITION

Inasmuch as five or six years have elapsed since the first edition of *Chemistry and Industry of Starch* was written, it seemed desirable to revise the text to include the important advances that have been made in this very active field during this interim. To do so has required enlarging many of the chapters and in some cases, rewriting the subject material.

A second edition, furthermore, provides an opportunity to meet the shortcomings which appear after publication of the first. In this case it appeared that the text could be improved by extending the discussions on the technology of starch, particularly in regard to manufacture and uses. Accordingly, the chapter on the manufacture of corn starch was rewritten and an additional chapter was included on miscellaneous uses which have assumed some importance in recent years. Another addition, written to fill an apparent void in the first, is a chapter on analytical methods that are most commonly used with, and in some cases developed specifically for starch or starch products. These are in addition to methods for inspecting the physical properties of starch, such as the colloidal characteristics of pastes, which are again given in Chapter VI.

All of these changes have resulted in an enlarged, and it is hoped, improved second edition.

Again thanks are due to the many workers who have continued to collaborate in reviewing, organizing and writing the subject matter of this important phase of carbohydrate chemistry. A special debt of gratitude is again acknowledged to the publishers, Academic Press, for their interest and support of publications in the field of biological chemistry in general and in starch chemistry in particular.

RALPH W. KERR

November 22, 1949
Argo, Illinois

PREFACE TO FIRST EDITION

The purpose of this text it to review and bring up to date our accumulated knowledge of starch and of the more important products derived therefrom. Considerable progress has been made in recent years in many phases of the subject, both fundamental and applied. The picture of the ultimate organization of the starch granule, of the chemical structure of starch molecules, and of their behavior in solution or dispersion, in particular, has been materially clarified by recent research. Undoubtedly, our knowledge of the starches is far from complete. The application of starch products in industry, particularly chemical industry, is still in its infancy. The beginner in carbohydrate chemistry should not, therefore, misinterpret our effort towards coordination by forming the opinion that further investigations are not required or that the subject has reached a static stage. Rather, it is hoped that he will see the possibilities for further fundamental research with a clearer vision and the possibilities for new industrial applications of this almost inexhaustible supply of raw material.

It is also hoped that this survey will be of benefit not only to the carbohydrate chemist but to the technologist, whose daily task is concerned with more efficient production of starch products and with their more efficient utilization. Because of these primary objects, the manner of presentation of the subject is that of descriptive chemistry. Possibly a kinship between the approach to the study of the substances encountered in elementary chemistry and the approach to the study of starch will be more readily apparent.

The text is not necessarily intended to be comprehensive. Full justice could not be done in one volume to many important divisions of the subject. Several of these topics, however, have been adequately discussed elsewhere and the reader is referred in the text to these works for further study. Representative, cardinal topics are discussed by those who are identified with the particular line of study. In respect to technology, we have attempted to present the American view-point and practice with greater emphasis, since this seems to have been neglected in earlier texts.

The writer desires to express his thanks and gratitude to those co-authors who have so graciously cooperated in writing the text, to his associates at the Corn Products Refining Company for their help and constructive criticism, to his wife, Dorothy E. Kerr, for her aid in preparing the manuscript, to Dr. W. W. Pigman for reading the text and to Mrs. Lucia Dawe for reading the copy and the proofs. Especial thanks are due the publishers, Academic Press Inc., who suggested writing this timely text and who have made its publication possible.

Argo, Illinois

RALPH W. KERR

CONTENTS

List of Co-authors v
Preface to Second Edition vii
Preface to First Edition ix

SECTION I. OCCURRENCE IN NATURE

Chapter I. OCCURRENCE AND VARIETIES OF STARCH 3
- 1. Introduction: Source of Starch 3
- 2. Size of Starch Granules 3
- 3. Shape of Starch Granules 4
- 4. The Hilum 5
- 5. Striations 5
- 6. Growth and Organization of the Starch Granule 7
- 7. Swelling of Granules in Water 10
- 8. Description of Individual Starches 14
- 9. Photomicrographs of Various Starches 17

SECTION II. PREPARATION

Chapter II. THE MANUFACTURE OF CORN STARCH 29
- 1. The Corn Grain 31
- 2. Cleaning and Steeping 37
- 3. Milling and Separation 47
- 4. Dehydration 58

Chapter III. MANUFACTURE OF MODIFIED CORN STARCHES 63
- 1. Starches Modified in Color 63
- 2. Odorless Starches 66
- 3. Mobile (Dry) Starch 67
- 4. Starches of High Viscosity 71
- 5. Starches of Reduced Viscosity 76
- 6. Cold Water Paste Products 81
- 7. Modification of Starch with Gluten 84

Chapter IV. MANUFACTURE OF TAPIOCA AND ARROWROOT STARCHES 87
- 1. Importance of Tapioca Starch in the United States 87
- 2. Raw Material 87
- 3. Production Methods 90
- 4. American Production 93
- 5. Arrowroot Starch 97

Chapter V. MANUFACTURE OF WHEAT, POTATO, AND OTHER INDUSTRIAL STARCHES 99
- 1. Wheat Starch 99
- 2. White Potato Starch 104
- 3. Sweet Potato Starch 108
- 4. Sorghum, Glutinous Sorghum and Glutinous Maize Starches 112
- 5. Sago Starch 114

CHAPTER VI. EVALUATION OF MODIFIED STARCHES IN PRACTICE
1. Viscosity of Hot Pastes
2. Cold Paste Body
3. Pasting Characteristics of Starches or Paste History
4. Alkaline Fluidity
5. Gel Properties
6. Plasticity in Pastes
7. Paste Shortness
8. Syneresis or Water Retention in Starch Pastes
9. Gelatinization Temperature of Starch
10. Protective Colloid Effect of Starch Sols
11. Supplementary Tests on Paste 148
12. Color of Starch 149
13. Odor of Starch 151
14. Mobility of Dry Starch 151
15. Miscellaneous Tests 151

SECTION III. PROPERTIES

CHAPTER VII. PHYSICAL PROPERTIES OF STARCH 157
1. Introduction 157
2. Physical Properties of Native Starch Granules 158
3. Crystalline Forms of Starch 159
4. Swelling, Gelatinization, and Retrogradation of Starch 165
5. Fractionation of Starch and Properties of Starch Fractions 167
6. Iodine Reaction with Starch 170
7. Optical Rotation of Starch 173
8. Other Aspects of the Colloidal Behavior of Starch 175

CHAPTER VIII. CHEMICAL PROPERTIES OF STARCH 179
1. Introduction 179
2. Composition 181
3. Structure 197
4. Non Carbohydrate Constituents of Starch 228
5. Synthesis 234
6. Conclusions 239

SECTION IV. REACTIONS

CHAPTER IX. THE HYDROGEN BOND IN STARCH AS A BASIS FOR INTERPRETING ITS BEHAVIOR AND REACTIVITY
1. Theory of the H Bond
2. Association in Starch
3. Hydration, Gelatinization, and Gelation in Terms of Changes in H Bonding
4. Disorganized Starches
5. Location of H Bond in Starch and Influence of Position on Behavior

CHAPTER X. DERIVATIVES OF STARCH
1. Introduction
2. Acyl Derivatives of Starch
3. Alkyl Derivatives of Starch
4. Nitric Acid Derivatives of Starch
5. Phosphoric and Sulfuric Acid Derivatives of Starch
6. Carbon Disulfide Derivatives of Starch
7. The Halogen Derivatives of Starch
8. Oxidized Derivatives of Starch

IAPTER XI. OXIDATION OF STARCH 325
1. Introduction 325
2. Historical 325
3. Oxidation by Hydrogen Peroxide 326
4. Oxidation with Halogens 327
5. Oxidation by Periodic Acid 328
6. Oxidation by Commercial Methods 336

IAPTER XII. DEXTRINIZATION 345
1. White Dextrins 345
2. British Gums 348
3. Canary Dextrins 350

CHAPTER XIII. MANUFACTURE OF DEXTRINS 357
1. Introduction 357
2. Torrefaction Dextrins Made with No Added Catalyst (British Gums) 357
3. Torrefaction Dextrins Made with Acid Catalysts 361
4. Torrefaction Dextrins Made with Basic Catalysts 366
5. Wet Dextrinization with Acid 367
6. Dextrinization by Enzymes 369

CHAPTER XIV. ACID HYDROLYSIS OF STARCH 375
1. Introduction 375
2. Starch As the Substrate in Hydrolysis 375
3. Reactions Involved When Starch Is Treated with Acid 377
4. Hydrolysis Reaction 379
5. Recombination 383
6. Intramolecular Dehydration and Other Destructive Reactions 386
7. Effect of the Substrate Starch on the Acid Concentration 390
8. Neutralization and Clarification 394
9. Secondary Catalysts 397
10. Commercial Methods for Acid Hydrolysis of Starch 398

CHAPTER XV. THE AMYLASES. PROPERTIES AND PRODUCTION 407
1. Introduction and Historical 407
2. Classification of Amylases 409
3. Types of Activity and Evaluation 410
4. Properties of Amylases 420
5. Production of Amylases 431
6. Other Enzymes with Amylase-like Properties 436

CHAPTER XVI. MODIFICATION OF STARCH BY ENZYMES 443
1. Introduction 443
2. Liquefaction of Starch 444
3. Dextrinization 449
4. Saccharification 450

CHAPTER XVII. MISCELLANEOUS REACTIONS 455
1. Reaction of Starch with Complexing Agents 455
2. Reaction of Starch with Formaldehyde and other Cross-Linking Reagents 466
3. Hydrogenation of Starch 472

SECTION V. USES AND ANALYSIS

CHAPTER XVIII. USES OF STARCH IN PAPER MANUFACTURE 483
1. Introduction 483
2. Beater Sizing 484
3. Calender Sizing 491
4. Tub Sizing 493
5. Surface Sizing: Clay Coatings 494

CHAPTER XIX. USES OF STARCH AND STARCH PRODUCTS IN THE FERMENTATION INDUSTRIES 515
1. Introduction 515
2. The Agricultural Situation 517
3. Prospects for New Markets 518
4. Ethanol 519
5. *N*-Butanol and Acetone 524
6. Glycerol 526
7. Lactic Acid 527
8. Butylene Glycol 528
9. Citric, Gluconic and Itaconic Acids 529
10. Penicillin and Other Antibiotics 530
11. Other Fermentation Products 530

CHAPTER XX. USE OF STARCH AND STARCH PRODUCTS IN FOODS 535
1. Introduction and General Discussion 535
2. Flour in Bread Making 536
3. Crackers and Biscuits 548
4. Salad Dressing 555
5. Starch in Baking Powder 558
6. Confections 559
7. Additional Uses 562

CHAPTER XXI. USE OF STARCH PRODUCTS IN THE TEXTILE INDUSTRY 569
1. Introduction 569
2. Requirements of a Warp Size 573
3. Sizing of Continuous Filament Yarn 579
4. Sizing of Spun Yarn 581
5. Finishing Textiles with Starch Size 582
6. Printing Pastes 585

CHAPTER XXII. STARCH ADHESIVES 589
1. Introduction 589
2. Types of Starch Adhesives 595
3. Manufacture of Adhesives 613
4. Conclusion and Acknowledgement 625

CHAPTER XXIII. MISCELLANEOUS USES FOR STARCH 627
1. Ceramics 627
2. Cosmetics, Pharmaceuticals and Insecticides 631
3. Detergents 633
4. Emulsifying and Dispersing Agents 634
5. Explosives 635
6. Laundry Starches 636
7. Mining and Metallurgy 639
8. Plastics and Coatings 652
9. Printing 653
10. Water Treatment 654

CHAPTER XXIV. METHODS OF ANALYSIS FOR STARCH
1. Quantitative Determination of Starch 659
2. Analysis of Starches [illegible]
3. Qualitative and Quantitative Determination of Starch Modifications 667

AUTHOR INDEX 677

SUBJECT INDEX 691

SECTION I. OCCURRENCE IN NATURE

Chapter I

OCCURRENCE AND VARIETIES OF STARCH

1. Introduction: Source of Starch. Starch is the chief reserve carbohydrate of plants. It is therefore one of the most widely distributed substances in the vegetable kingdom. For example, it occurs in seeds and fruits as a relatively permanent reserve to be drawn upon by the young embryo when the seeds or fruits are planted. In such cases it is usually found accompanied by other reserves, such as proteins, fats, and organic phosphates. Starch is found in the vegetative parts of plants, such as tubers, the living cells of the pith, and in the cortex of roots and stems. Starch is also found in the latex of certain plants, and it may be found in transitory form in green leaves. A substance closely related to starch, particularly to some varieties of starch, is found in the liver and other tissues of higher vertebrates as a temporary reserve carbohydrate. It is called glycogen or animal starch.

In parts of plants where starch is stored as a more permanent reserve the amount present may be quite large. This is especially true of some seeds or grains, which may contain as high as 70% starch. To a lesser extent it is true of tubers and roots and the pithy stems of certain palms, which may contain 25 to 30% starch.

Although starches from other sources have an academic interest in the rounding out of a study of the problems involved in starch chemistry, only those that can be obtained in high yield from plants which grow or are cultivated in abundance, such as cereal grains, roots, and tubers, are of industrial importance at present. The work reported here is concerned chiefly with a discussion of these latter varieties.

Starch granules from different sources show much variety in shape and size and other superficial physical characteristics. Microscopic examination furnishes considerable information not only for the determination of the origin or variety of an unknown sample but also on given types of starch, which may aid in a fuller understanding of its behavior.

2. Size of Starch Granules. Starches from different sources show a wide range in average size. Starch granules from canna and white potato are among the largest; those from rice and buckwheat are among the smallest. While it has long been known that the larger granules of any particular type of starch, such as corn, gelatinize more easily than the smaller granules (1), it would now appear that there may be some correlation between the dispersibility of a type of starch and its average granule size. Pacsu (2) has arranged a series of starches according to average granule size and reports that not only does the heat of gelatinization decrease with average granule size for the series consisting of potato, tapioca, wheat, corn, and rice, but also that the maximum consistency of paste obtainable in pyridine-water mixtures falls off with decrease in granule size.

Generally, the size of starch granules is expressed as the length of the longest axis, in microns. The sizes of the largest and smallest granules are noted, and the result for a given sample expressed either as a range or as an average size. Some starches, such as canna or rice, show granules which differ little in size from the mean. In these cases the average granule size is a significant value. Other starches, such as maize, show quite a range in granule size, while still others, rye and wheat starches, are composed partly of relatively large and partly of very small granules. Starch granules vary in size from about 2 μ to 150 μ.

3. Shape of Starch Granules. Of less significance is the actual shape of starch. From an examination of the variety of shapes from any one type, as for example corn starch, it is apparent that the final shape depends to a large extent on the environment surrounding the growing starch granule. Those of corn starch, which are laid down in a glutinous matrix, so to speak, become compressed as the grain matures and the gluten dehydrates. The result is a horny variety of starch, very angular in shape and highly refractive (Fig. 13). At the other extreme, starch which is deposited in the crown of the corn kernel is very loosely packed and easily separated out by mechanical means. This type is referred to as floury and under the microscope appears to be perfectly round (Fig. 14). These latter granules are relatively fragile in comparison with the horny type and are easily injured during milling.

In general, starches formed under continuously higher moisture conditions and in the absence of horny, glutinous substances tend to be larger, more regular in shape, and more fragile. Examples are the canna, the potato, tapioca, and arrowroot. At the other extreme we have horny corn starch, millet, and rice starch.

The descriptive terms applied to the former types are (*a*) round, (*b*) elliptical, or (*c*) oval; to the latter, (*d*) polygonal.

The polygonal shape, as intimated above, probably results from close packing. Occasionally the crowding together of granules is such that separation into individual granules is not complete in the process of milling. Such aggregates of two or more granules are referred to, although erroneously, as "compound granules." True compound granules are rare and are probably another of nature's freaks like the double yolks in eggs. Occasionally the more rounded granules grow in such proximity to each other that, before milling, compound granules may be observed. When these compound granules are separated in milling the starch, a fifth form is observed, called kettle shape or (*e*) truncated.

4. **The Hilum.** This spot on the granule, clearly discernible in some starches, is said to be the organic center or nucleus around which the granule has grown. It may correspond with, but more ~~frequently does~~ not, the geometric center of the granule.

The hilum is more precisely located by observing starch granules under polarized light. One theory of granule composition suggests that the density and distribution of moisture throughout the granule are not uniform. In the growing granule the hilum contains more water and is softer than other regions. As the granules dry out, stresses develop within the stratified structure of the granule, which results in the latter becoming brilliantly illuminated in polarized light, with the exception of two dark lines which intersect at the hilum. Normally these lines form a cross (Fig. 16), but as the hilum tends to be more eccentric the X-shaped cross approaches the form of a V (Fig. 17). As granules tend to become overdry, or take on excessive water, as in gelatinization, the refraction is lost; this is evidence that pressures have been relieved or equalized. In the former case the refraction of the granules may be increased or restored by remoistening.

These facts have been interpreted differently in recent years. X-ray powder diffraction patterns of starch suggest a crystalline lattice within the granule. Since these patterns change with moisture relationships, it would seem that the elements of water enter into the crystalline structure. Anisotropy may therefore be due to a crystal-like structure within the granule.

Opposed to the explanation that birefringence is due to crystallinity is the observation that one may be destroyed and not the other. Brimhall (3) reported that starch granules which had been severely dried, as in the production of pyrodextrins, looked like those of the native starch and were still strongly birefringent. However, the crystalline X-ray pattern had given way to a practically amorphous one.

Whatever the ultimate explanation may be, it would seem that as some starch granules dehydrate during maturity or the drying operation in normal starch manufacture this dehydration is of sufficient non-uniformity so that cracks appear on the surface of the granule. These are visible in ordinary light and seem to originate from the hilum.

The location of the hilum, by an inspection of characteristic fissures, or by observation of the darkened cross in polarized light, is helpful in characterizing the various starches.

5. **Striations.** By careful examination, under the microscope, some

starches show a series of markings arranged concentrically around the hilum; these are called "striations." In some starches, such as that of the potato, these markings are quite plain. In others, it is necessary to stain the granules with dilute chromic acid to render them visible, while in still others there is apparently no evidence of these supposed lamellations whatsoever. These markings are best seen, when present, by oblique illumination and careful focusing. Rarely at one time are more than a few granules in the field in sufficiently sharp focus to show the striations.

It was formerly thought that the striations were significant clues to the organization of the granule. It would now seem that these markings are superficial. For example, where they are most pronounced, as in potato starch, one might expect that if sufficient force was applied to crush the granule, it might tend to fracture first along parallel lines. Figs. 30 and 31, however, show that the starch fractures most easily along radial lines. Moreover, the striations should not be assumed to be necessarily connected with the finer, physical organization of the granule; *i.e.*, its molecular arrangement. This is discussed in the next chapter.

It may be that the number and arrangement of the striations are the result of and related to some rhythmic force or set of fluctuating conditions of botanical growth. While it has been established that these markings do not correspond to definite intervals of solar time in the growth period of the plant from which they are obtained, one of the most logical explanations for their existence is that they represent periods of relative inactivity of deposition of starchy material either by apposition or by intussusception, as the case may be. If granule growth is by the former process, then the outermost layer is the last deposited. If growth is by intussusception, then the outermost layer is the oldest. This latter view would be more in accord with the fact that the outer layer appears to be more dense, less hydrated, and least susceptible to enzymic attack than any other region of the granule, particularly in such starches as corn starch. Ling (4) has claimed that the original, outermost layer of some starches, such as potato, is lost in milling, but may be found with the pulp residue.

Whichever is the manner of growth, as layers of dissolved constituents are deposited, followed by periods of inactivity, it is supposed that the surface of these layers either agglutinates or condenses, or continues to increase in molecular magnitude or to be modified chemically into a more impervious membrane, which latter resists re-solution of the newly deposited granule layers. This action is possibly the result of contact with the cell sap, its hydrogen ions, metallic ions, its enzymes, or some unknown stimulus.

In some instances, a comparatively large amount of re-solution may take place when these membranes (used in the colloidal sense) do not form. In other cases, even though they do form, their permeability may remain high and their dispersibility in water may not decrease because of a high concentration of ions in the cell sap which favors the retention of permeability.

6. Growth and Organization of the Starch Granule. The growth and organization within starch granules have been the subjects of a countless number of researches. Reichert (5) has reviewed the earlier literature on this work. Several dissertations have been given in recent years, particularly by Badenhuizen (6, 7), Frey-Wyssling (8) and Alsberg (9). Sandstedt (10) has reviewed the recent work in this field.

The synthesis of starch and the development of starch aggregates in plants and particularly in seeds, are apparently far more complicated processes than might be anticipated. Biochemical synthesis will be discussed elsewhere (Chapter VIII). The role of the amylases, now relegated to a position of secondary importance in the development of starch substance, with the discovery that synthesis of the polymer takes place by means of the enzyme phosphorylase, is still none too clear, but may be surmised from recent work of Sandstedt and others.

Sandstedt has shown that the development of starch in mature grains proceeds by several disinguishable phases. First of all, minute starch granules were found to be present in the unfertilized wheat ovary. Therefore, there are already present, and donated by the mother plant, the necessary chemical nuclei for phosphorylase activity, even before birth of the new embryo. These granules grow, after pollination, with the development of the kernel, and are very noticably larger on about the third or fourth day. Indeed these granules, which are found in the pericarp, reach a maximum diameter at about the fifth day. However, an enzyme is present in the pericarp which digests them (11). Evidence of attack is visible at about the fifth day and pericarp starch has practically disappeared by amylase action by the 16th day after fertilization. At about the fifth day another type of starch granule begins to develop and this was found to reside in the endosperm. These develop rapidly from a very small, bean shaped granule into a large lenticular shape. At about the time the kernel attains full length, or about the 12th day, a third type of granule begins to develop in cells which contain lenticular granules. These are spherical in shape, very small in size, and completely fill the spaces between the large lenticular granules. Thus the several stages of synthesis, amylolytic degradation, and resynthesis indicate that the disposition of starch substance as it is found in the mature seed is an involved series of processes.

The early appearance of pericarp starch and its eventual disappearance were also indicated by the work of Brenchley (12), Percival (13) and Bice *et al.* (14). The latter also noted a progressive increase in the size of starch granules during maturation, and this was taken by Sandstedt to support the theory of growth by apposition. Indeed, Sandstedt presents data to show that embryonic granules, which appear as bean shaped particles in the endosperm, may become the "nuclei" of the large lenticular granules that develop later.

Sandstedt has attributed the greater birefringence and greater iodine staining power of the center of wheat starch granules to the type of molecule laid down in this region rather than to differences in density or polymer-polymer association and has pointed out that many waxy starches have a center which stains blue with iodine. However, in the case of wheat starch granules Bice *et al.* have noted that there is a measurable and progressive increase in amylose content of starch which occurs in samples taken between the 8th and 24th day after pollination. Of course, this indicated change in average composition does not necessarily indicate that the composition of individual granules has changed during growth. The change may be explained by the development of a high-amylose-bearing granule, in increasing numbers, such as the small, spherical granules discussed above, which begins to make its appearance at about this time. But Bice *et al.* could find no difference between small and large wheat starch granules in respect to their amylose to amylopectin ratio. These workers proposed that the granules in the peripheral regions of the endosperm are richer in amylopectin.

However, it is from these regions where not only the lenticular granules are originated early in the development of the kernel, but also the spherical granules in the latter stages. Sandstedt has confirmed and extended the observations of Gordon (15), and Mottier (16) and has shown that the aleurone is the meristematic tissue which produces endosperm cells. Apparently, aleurone cells divide both tangentially, producing daughter starch cells, and radially, producing new aleurone cells. Evidence was presented to show the development of starch cells from the periphery inward and to show that the aleurone layer continues to form new starch cells until so late in the growth of the kernel that the last-formed cells do not complete their development before the kernel matures.

No material difference has been found by the writer between the amylose to amylopectin ratio of large and small corn starch granules when determined by iodine spectrophotometry.

Amylopectin, the branched component of starch, is primarily responsible for the organization and crystallinity of the granule, according to Meyer and Bernfeld (17). Therefore, it might be expected to be fairly well distributed throughout the granules of the more common varieties of starch, since it is the framework which extends into all regions of the granule. These workers visualize starch as comprised of concentric layers, in each of which highly ramified amylopectin molecules are folded and interwoven into a reticulated network. The linear portions of these branched molecules are oriented in a general radial direction with respect to the granule, and secondary valence forces, such as hydrogen bonding, operate tangentially to impart mechanical coherence to these substructural units. Accordingly, certain areas within the granule may be highly organized, consisting of a micelle which is composed of many parallelwise oriented linear sections of molecules. Only parts of an amylopectin molecule may be in one such micelle and the balance of the molecule may extend into less oriented or amorphous regions and from these into a second or several other micelles. The region between micelles is loosely woven with randomly placed molecules, or sections of molecules, and is relatively amorphous.

It would appear that a considerable portion of the amylose, or linear polymer, particularly the short, unbranched chains, cannot be very firmly held, or oriented within the micelles, since by treating starch with hot water, and thus effecting a partial swelling of the framework, the amylose is dissolved and diffuses readily from the granule. A part of the amylose fraction of starch is not solubilized, even at boiling temperature in the case of corn starch, and is retained within the structure of the granule, possibly owing to the fact that these structures are sufficiently branched so as to have entered into the framework, or structural part of the granule in a manner analogous to amylopectin. In the case of corn starch at least, the insolubility of this fraction is not due to greater chain length alone since longer amylose chains may readily be dissolved from potato and tapioca starches by hot water extraction at a temperature considerably less than required to leach the shortest chains from corn starch.

Meyer (17) has pointed out that the reticulated network structure of amylopectin molecules provides an explanation for the swelling of starch granules without dissolving. The granules swell because the tangential secondary valence bonding is weakened by hydration. However, the crystallites do not readily disperse but are held together in the form of a swollen granular mass by parts of amylopectin molecules that tie the micelles together. In some starches, the network survives to some degree even up to 120°C. Although a granular organization, consisting of concentric layers of radially oriented "trichites," as proposed by A. Meyer (18) and shown in Fig. 1A, is confirmed by the studies of K. H. Meyer and coworkers, a necessary qualification is added that the crystallites are held together by

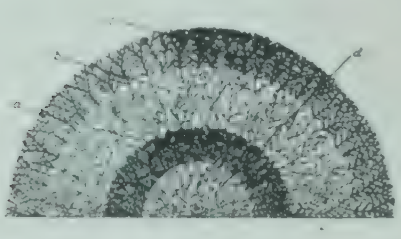


FIG. 1A. Trichite structure of the starch granule according to A. Meyer (18).

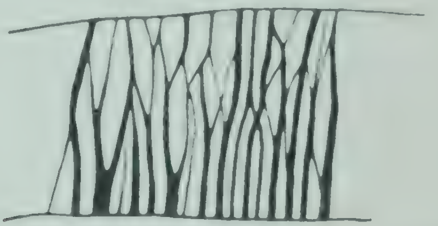


FIG. 1B. Scheme of the fine submicroscopic structure of a starch granule, according to Meyer and Bernfeld (17).
Fine strokes are chains or parts of individual chains, and heavy strokes are parts of micelles, or radial bodies in a layer of the granule.

free parts of chains, through primary valences, which constitute an amorphous and elastic binding material. Such a structure is represented in Fig. 1B, according to K. H. Meyer.

7. Swelling of Granules in Water. In the natural state starch is insoluble in cold water but appears to absorb about 25 to 30% and does not swell appreciably. With rising temperature, the hydrogen bonds that hold the micellar structural units together and the solvent water molecules in an aggregated state tend to dissociate. The smaller, dissociated water molecules, at a higher energy level, are able to permeate the weakened starch structure and gradually hydrate the many hydroxyl groups along the length of the starch molecules. At 60°C., according to Meyer and Bernfeld (17), corn starch takes up about 300% water, and at 70°C., about 1000%, based on its original weight. At the point of maximum swelling the granule can take up 2500%, so that not more than 4% of dry substance is present.

During swelling the free molecules of amylose and some free molecules of amylopectin of low DP dissolve and leave the granule by diffusion Finally there is left the much less soluble structural part of the starch which surrounds water, or a very dilute aqueous solution. In some cases, such as for example, potato starch, the exterior layer of the granule may, according to Meyer and Fuld (19), detach itself from the interior of the granule thus forming a membrane which encloses a watery interior. Badenhuizen (20) recently reported that he often observed the separation of the peripheral layers, leaving intact nuclei behind, whereas in the case of some starches, for example, tapioca, the layers remain firmly connected and each granule develops one single vesicle in the usual way.

The elimination of amylose by diffusion lessens the osmotic pressure of the interior, according to Meyer and Bernfeld, and swollen granules maintained in water in excess of 80°C. slowly contract. Of course, severe agitation, pumping at high pressures, passing between heated rollers, or other methods of handling in practice which involve the application of high shearing stresses to these distended granules may result in their rupture, particularly if the granules have been additionally weakened by chemical treatment.

Meyer and Fuld have concluded that the principal cause for paste viscosity of all kinds of starches is the hydrodynamic effect produced by the swollen granules in suspension, and have attributed the greater viscosity of potato starch, compared, for example, to corn, to the fact that swollen potato starch granules behave like vesicles filled with water and are semipermeable towards aqueous solutions, whereas the swollen granules of corn have a more compact structure.

Cox and MacMasters (21) found no relationship between the stretching ability of various starch granules such as waxy maize, tapioca, corn, and potato, and their paste viscosities at 95°C. Neither was there a correlation between plasticity of the pastes and stretch of the granule. These investigators found that individual granules could be pulled away from the main body of 3% corn starch pastes with ease, whereas potato starch granules underwent more stretch before separating. It was difficult to separate single granules from a 4 or 5% corn or potato starch gel.

Meyer and Bernfeld (17) have observed that when starch swells in a sufficient quantity of water, as for example, 40 to 50 parts, the envelopes of amylopectin become separated and are deposited, while the amylose diffuses into solution. When the quantities of water are much smaller, the envelopes contract against each other and some envelopes penetrate into others, and become entangled. Meyer and Fuld therefore believe that the action involved in the formation of a starch paste is analogous to the action when surfaces of non-vulcanized rubber come into direct contact. Large molecules protrude from one surface and extend into and beyond another surface which is in contact.

In the opinion of the writer the formation of paste, and more particularly gel, from incompletely dispersed starch granules, and the character of these phases, is additionally dependent to a very large degree upon the amount and character of the amylose (and amylopectin in the case of some starches) which has dissolved into the external aqueous phase and which may, or may not, depending on its structure and size, create rigid, relatively strong connecting bridges that tie the granule residues together. A paste made by thorough extraction of corn starch at very high dilution with water at 80°C., and by concentration of the insoluble residues into the consistency of a paste by filtration is quite different in character from paste made at equal solids concentration by heating for an equal time at 80°C., but from which the soluble amylose in the external phase has not been removed.

A study of the manner of swelling of starch granules by use of the microscope, is frequently used as an additional aid to distinguish the various types of starch and occasionally to furnish a clue as to the history of its manufacture. Application of this technique has been described by Sjostrom (22). Frequently, starch products are obtained in practice that are so modified in physical appearance that identification is almost impossible by usual methods of observation. These are usually specimens in which the granules are in various stages of gelatinization and are often mixtures of starch, such as corn mixed with wheat starch. The characteristic manner in which the various granules swell being known, it is less difficult to make an identification. Some of the peculiar properties which distinguish one starch in swelling from another have already been mentioned. Many others will be found in the work reported by Sjostrom. Several of these will be reviewed which may serve to amplify the discussion of granule organization given above.

Fig. 18 shows the very first stage of swelling of rye starch at 55°C. in water. This picture, taken at a magnification of 450, is, according to Sjostrom, visual confirmation of a granular organization similar to the trichite structure (Fig. 1A) proposed by Meyer (18). Organization is definitely along radial and concentric lines, and it might be supposed that the microstructure of the granule would reflect its submicrostructural organization.

Figures 19 to 26 show the early stages of swelling of potato, sweet potato, tapioca, canna, sago, and rye starches at 65-70°C. in water. According to Badenhuizen (5) swelling is tangential exclusively, the lamellae confluxing finally to form the outer sac of the swollen or pasted granule. The growth of this sac is not due to internal pressure according to this worker. Rather, the internal layers are pulled asunder by the peripheral ones. This would account for the apparent free space often seen in the center of granules as they start to swell. Figure 19 shows one such potato starch granule with an apparent free space. Swelling does not appear to be due to peripheral forces alone, and the final sac does not appear to be formed by the confluxing of concentrically arranged layers of the starch. Rather the organization is both radial and concentric and smaller units form within the granule, with each of these units showing its own striations. The finer organization of these subgranule units is shown in Figs. 20 and 21 when the potato starch, before forming a paste, has been mildly treated with acid to weaken organization along concentric lines (see below).

Figure 22 for canna starch and, more particularly, Fig. 23 for sweet potato starch show granules at the instant the internal pressure has exceeded the elastic limit of the outermost layers at some part, according to Sjostrom, and the granule virtually explodes in this region. Furthermore, it is obvious, in the case of the sweet potato starch, that hydration and swelling have been retarded in one section of the granule and that it has not, therefore, proceeded uniformly in respect to the concentric layers. It should be remembered that these starches were photographed in the early stages of swelling, not the final. They show differences from some of the other starches, such as corn starch (Fig. 27). Figure 25 illustrates the peculiar curled shape assumed by the granules, such as rye starch, in heated water above 65°C. Curling is characteristic of both wheat and rye and has been used to identify these starches in the later stages of swelling.

Some treatments used in starch manufacture, such as dry heat, weaken the organization of the granule more along concentric lines, as noted by Sjostrom (22) and Badenhuizen (5, 23). But other treatments, as with weak acid, apparently weaken the structure more along radial lines (22). Figure 28 illustrates the initial phases of swelling of a torrefaction dextrin from corn. The gradual loosening of concentric layers is clearly shown. Figure 29 shows the radial cracks that develop when an acid-modified, thin boiling starch is heated in water to 95°C. The acid treatment has definitely reduced the tendency of the granule to swell. Sjostrom interprets this to mean that the outer layers have become less elastic and will not swell and yield to increasing pressures. The granule cracks into smaller botanical units, and a lowered paste viscosity results.

In view of the discussion presented earlier, the results obtained with acid treated starches may be interpreted as follows: It would appear that the acid had penetrated the more amorphous regions of the granule and had hydrolyzed some of the connecting links—parts of amylopectin molecules—which held together the crystallites. When a sufficient number of these linkages have been broken, then when the starch is heated in water, visible aggregates of these micelles, or crystallites, may break away as the starch starts to gelatinize, owing to the lateral swelling in the lamellae. The fractures then occur parallel to the general direction in which the crystallites are oriented.

Proof that the organization which resists inibition of water and swelling extends along radial lines is obtained by crushing starch granules with pressure. Figure 30 shows the radial cracks that develop, extending towards the hilum, when potato starch in water is pressed between two glass plates. The effect may be exaggerated by using an acid-modified potato starch as shown in Fig. 31. Suspended in water, the units remain well defined and show practically no tendency to swell. Reference to Fig. 14 shows round and more fragile types of corn starch granules fractured, during manufacture, along radial lines but with no tendency to swell in cold water.

On the other hand if starch is ground as in a ball mill, so that eventually radial lines of cleavage are disturbed, as well as concentric, this starch will swell in cold water as shown in Fig. 32.

8. Description of Individual Starches. The following brief summary of the more common individual starches met with in industrial practice is given. Included is a discussion of several starches which, although they have not as yet assumed commercial importance except locally, are no doubt destined to be of great industrial value in replacing certain foreign starches which are not obtainable in periods of disruption of world markets. These are the sweet potato starch and the waxy starches, particularly waxy maize and waxy sorghum, which, as the following chapters show, may be used within limits when the use of a non-cereal starch such as that of sago or tapioca is indicated.

The manufacture of sweet potato starch is being developed at the present time in the southern sections of the United States under the original sponsorship of the government. Several waxy maize hybrids have been developed which show promise of cultivation for the industrial manufacture of starch. Leaders in this development have been the groups at the Iowa State College of Agriculture and at the University of Wisconsin. The industrial application of waxy sorghum starch has been stimulated by the work at the Universities of Nebraska and Kansas.

A description of the various starches is given in the order of their industrial importance in America. This is followed by photomicrographs arranged in the customary order of granule size.

a. Corn Starch (Maize). Corn starch is composed of granules of two shapes, depending on whether they are derived from the crown or floury regions of ordinary corn grains or the horny portion of the endosperm. Some species which are floury in type, such as Mandan corn, contain practically all round-shaped granules (Fig. 14). Horny species, such as Kutias, contain practically all polygonal-shaped granules (Fig. 13). Each type shows variations in size, but the average for both is about 15 μ. Starch of modern manufacture will show a small proportion of very small granules of the order of 5 μ. The upper limits of size are usually about 25 to 26 μ.

Compound granules are extremely rare. When observed in the corn grain, the granules exhibit a large circular hilum, but in a milled, dried starch the hilum is replaced by what appears to be a cavity from which fissures radiate. Striations are absent. The polarization cross is quite distinct and appears at the hilum or geometric center.

b. Tapioca Starch. The granules of *tapioca* or *cassava* starch average about the same size as those of corn starch; however, they differ in appearance from the latter in that they are round or oval in shape with an indentation on one side which is characteristic of this starch. Apart from the truncated shape, with an occasional conical pit extending almost to the hilum, this starch has very few characteristic features. The granules are softer than those of corn starch, and their structure is much less rigid and compact. The hilum is centric with some fissures. No striations can be seen. In polarized light a well defined cross is observed. The size of the granules varies from 5 to 35 μ with an average of 20 μ.

c. Potato Starch. The granules of *potato starch* vary greatly in size, from 15 to 100 μ. The largest are egg-shaped and, because of pronounced concentric striations, have the appearance of oyster shells. The hilum can be distinctly seen as a dot or short line eccentrically situated in the smaller end of the granule. The polarization cross is distinct and irregular. The small granules representing early stages of development are round or oval-shaped with only faint striations or hilum.

d. Wheat Starch. The *wheat starch* granules, like those of rye and barley, are either large or small, with few granules of intermediate size. The granules of wheat starch are thin and of elliptical or round shape with no distinct striations. A faint *centric* hilum can be seen in the larger granules (varying from 20 to 35 μ), whereas the smaller granules (from 2 to 10 μ) show no hilum. By polarized light some granules show faint indistinct crosses at the center; others show no lines. Sjostrom has characterized wheat and rye starches by the observation that at lower temperatures (65-70° C.) the granules swell into a bag-like shape, but near the boiling point they assume a peculiar and characteristic curved shape.

e. Sago Starch. Sago starch belongs to the group of starches with a large granule. The granules vary from 20 to 60 μ, are oval or egg-shaped, and resemble arrowroot and medium sized potato granules. Some appear as truncated ovals. Sjostrom has identified sago and arrowroot granules as bag-shaped with smooth outlines on gelatinization. This condition is maintained even at boiling temperatures. He has distinguished sago from arrowroot in that the former in this swollen state nearly always exhibits a well defined crater-like opening at the end of the granule. The swollen bag-like granules of the latter starch are much more irregular in outline and the openings are large, with indefinite contours.

The granules vary in size from 15 to 65 μ with faint concentric striae on the larger and more fully developed ones. The hilum is eccentric, and the polarization lines cross it in irregular patterns.

f. Arrowroot Starch. The average granule size and shape of *arrowroot starch* are similar to those of sago, but vary according to the source. Some varieties show numerous truncated granules but these latter are quite possibly related to "Brazilian arrowroot" or *Manihot.* Arrowroot granules from *Maranta* roots are simple, and range in length from 15 to 70 μ. Most *Maranta* of commerce consists of granules of fairly uniform size between 25 and 50 μ in length. Striations are usually faintly concentric with a centric hilum at which distinct polarization lines intersect. The hilum is located by a characteristic, double curved fissure.

g. Rice Starch. The *rice starch* granules are the smallest of the starches of commerce; their size is from 3 to 8 μ. The large granules are about the same size as, but more angular than, the smaller ones of corn. Their shape is definitely polygonal and they are sometimes found honeycombed in clusters or "compound" granules consisting of numerous component granules, the outline of the whole being either round or polygonal. Because of its small size, the characteristic features of the granule such as hilum, striations, and lines by polarized light are not distinct.

h. Barley Starch. Generally *barley starch* is found as a mixture of large and very small granules. These large and small granules are round or elliptical and measure from 20 to 35 μ for the former and 2 to 6 μ for the latter. The hilum, fissures, and striations are indistinct even with the large granules. Under polarized light the granule usually appears uniformly illuminated, with no lines.

i. Waxy Sorghum (Red Leoti). The size and shape of the *waxy sorghum* starch granule, particularly that of the Red Leoti strain, are very similar to those of corn and waxy maize. The average granule size is 15 μ, with extreme sizes of 6 and 30 μ. The hilum is centric and distinct with radial fissures. Striations are indistinct. The black lines by polarized light appear as regular crosses.

This starch, like that of waxy maize, can be distinguished from common corn by the formation of a reddish brown color with iodine.

j. Sweet Potato. The most common form of the *sweet potato* granule is polygonal and refractive, with the predominating smaller granules appearing similar in size to those of corn (10 to 25 μ). The hilum is centric and distinct, but striations are faint, if any. The black lines by polarized light are distinct and cross at the hilum.

k. Waxy Maize Starch. The starch granule of the *waxy maize* hybrids now grown is similar in appearance to that of common corn with respect to size, shape, and characteristic markings. The size range is from 5 to 25 μ, with distinct centric hilum. The polarization cross is also distinct and regular. Similar to waxy sorghum, waxy maize is distinguished from corn by its reddish brown coloration with iodine.

9. Photomicrographs of Various Starches.* Photomicrographs of various starches commonly encountered in practice are presented in the following pages, 18-25. Included also are those of the newly developed waxy starches, maize and sorghum. Photomicrographs of starches in various stages of gelatinization are also given. It should be pointed out that in all cases gelatinization was accomplished in aqueous media, with heat where specified, and not by the use of chemical swelling agents. The mechanism of gelatinization may not necessarily be the same in the two instances. Unless noted to the contrary, all photomicrographs were taken at a magnification of 200.

BIBLIOGRAPHY

1. Cook, D. H., and Axtmayer, J. H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 226 (1937).
2. Pacsu, E., and Mullen, J. W., *Ind. Eng. Chem.*, **34**, 807 (1942).
3. Brimhall, B., *Ind. Eng. Chem.*, **36**, 72 (1944).
4. Ling, A. R., in Comprehensive Survey of Starch Chemistry, ed. by Walton, R. P., New York, 29 (1928).
5. Reichert, E. T., The Differentiation and Specificity of Starches, etc., Part I, Carnegie Institution, Washington, D. C. (1913).
6. Badenhuizen, N. P., *Rec. trav. botan. néerland.*, **35**, 559 (1938).
7. Badenhuizen, N. P., *Protoplasma*, **32**, 440 (1940).
8. Frey-Wyssling, A., *Z. Botan.*, **33**, 362 (1938).
9. Alsberg, C. L., *Plant Physiol.*, **13**, 295 (1938).
10. Sandstedt, R. M., *Cereal Chem.*, **23**, 337 (1946).
11. Sandstedt, R. M., and Beckord, O. C., *Cereal Chem.*, **23**, 548 (1946).
12. Brenchley, W. E., *Ann. Botany*, **23**, 117 (1909).
13. Percival, J., The Wheat Plant, Duckworth and Co., London (1921).
14. Bice, C. W., MacMasters, M. M., and Hilbert, G. E., *Cereal Chem.*, **22**, 463 (1945).
15. Gordon, M., *Proc. Roy. Soc. Victoria*, **34**, 105 (1922).
16. Mottier, D. M., *Ann. Botany*, **35**, 349 (1921).
17. Meyer, K. H., and Bernfeld, P., *Helv. Chim. Acta*, **23**, 890 (1940).
18. Meyer, A., Untersuchungen über die Stärkekorner, Jena (1895).
19. Meyer, K. H., and Fuld, M., *Helv. Chim Acta*, **25**, 391 (1942).
20. Badenhuizen, N. P., *Trans. Faraday Soc.*, **42B**, 255 (1946).
21. Cox, M. J., and MacMasters, M. M., *Plant Physiol.*, **21**, 459 (1946).
22. Sjostrom, O., *Ind. Eng. Chem.*, **28**, 63 (1936).
23. Badenhuizen, N. P., *Chronica Botanica*, **2**, 123 (1938).

---

*Figures 1 and 16 to 32 are through the courtesy of O. Sjostrom and Industrial and Engineering Chemistry.

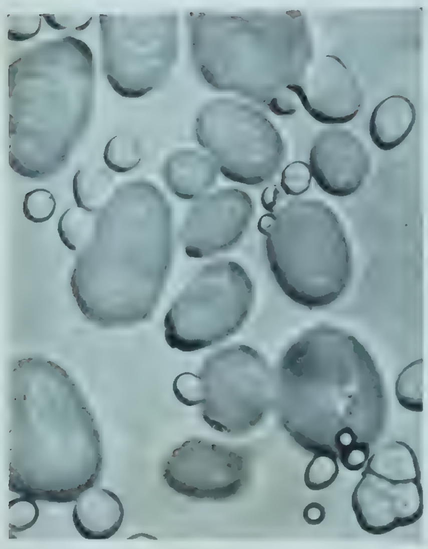


FIG. 2. Potato starch.

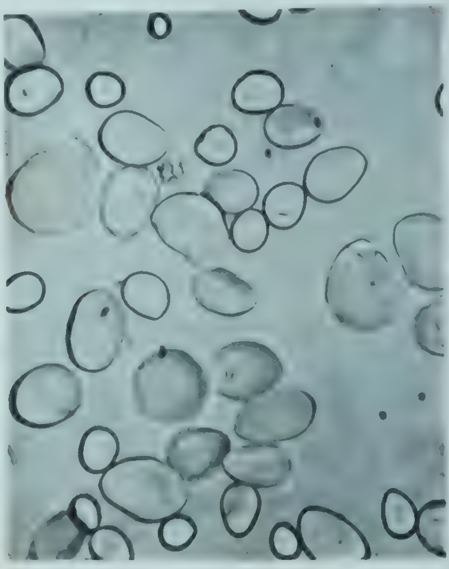


FIG. 4. Arrowroot starch.

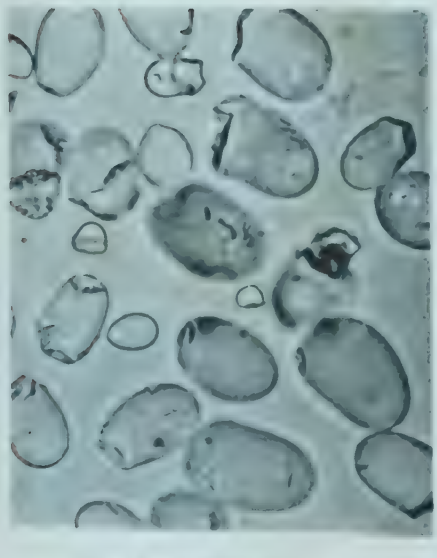


FIG. 3. Sago starch.

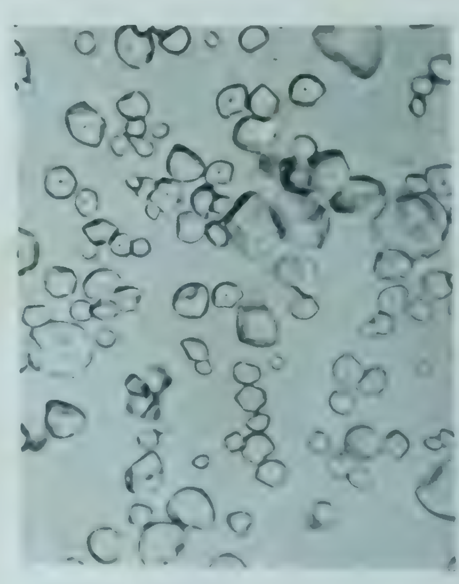


FIG. 5. Sweet potato starch.

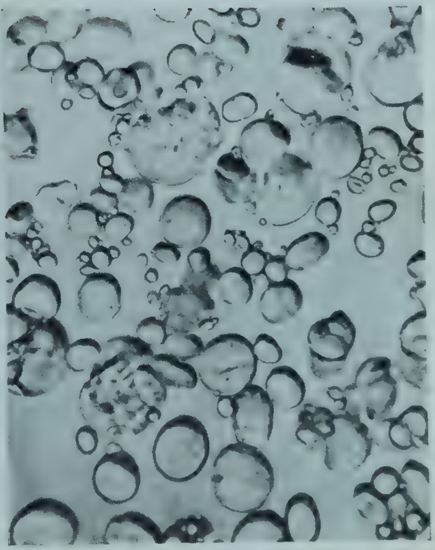


Fig. 6. Wheat starch.

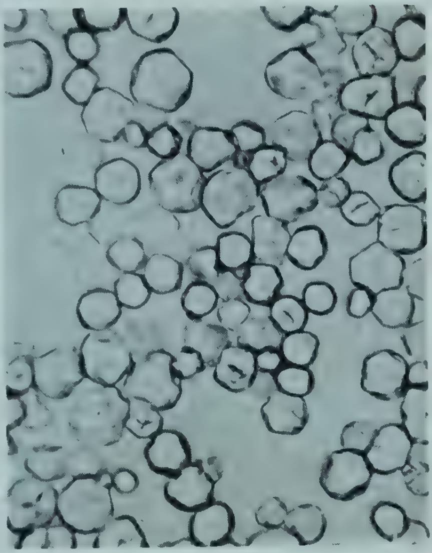


Fig. 8. Waxy sorghum starch.

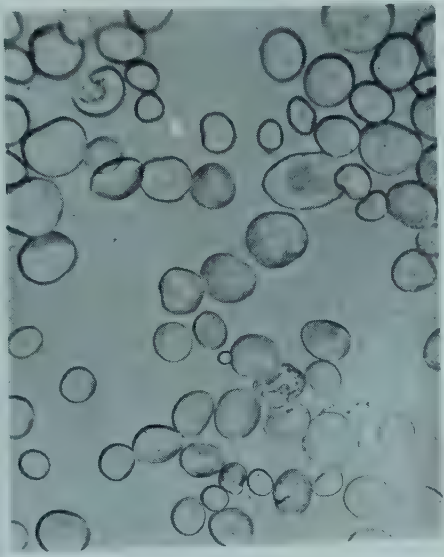


Fig. 7. Barley starch

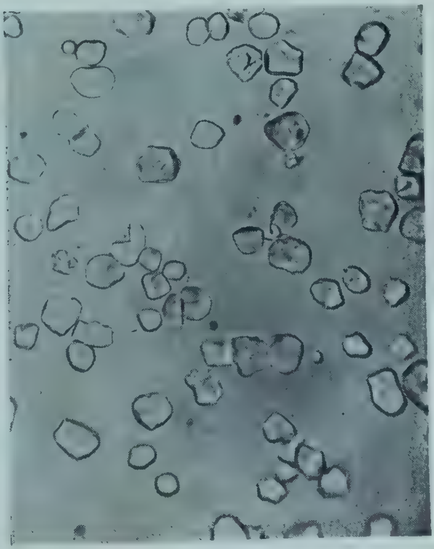


Fig. 9. Waxy maize starch.

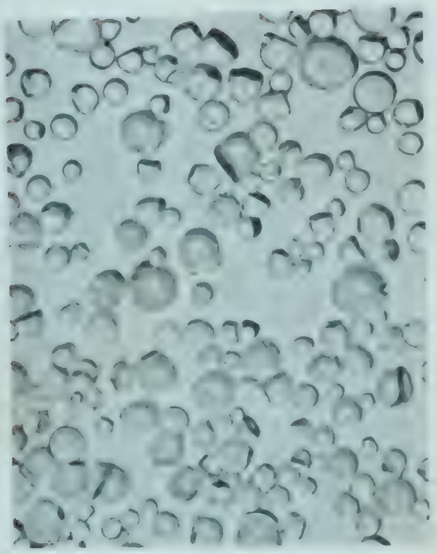


FIG. 10. Tahiti arrowroot starch.

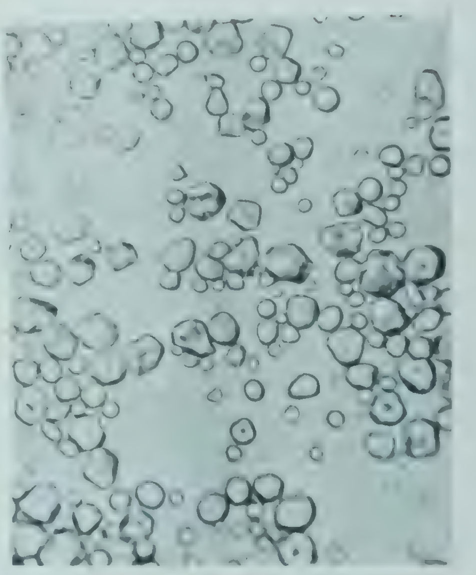


FIG. 12. Corn starch.

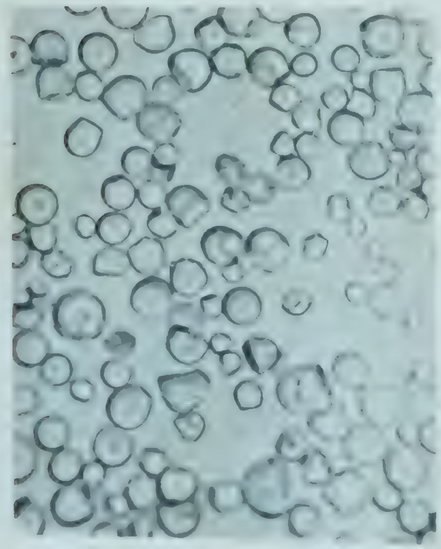


FIG. 11. Tapioca starch.

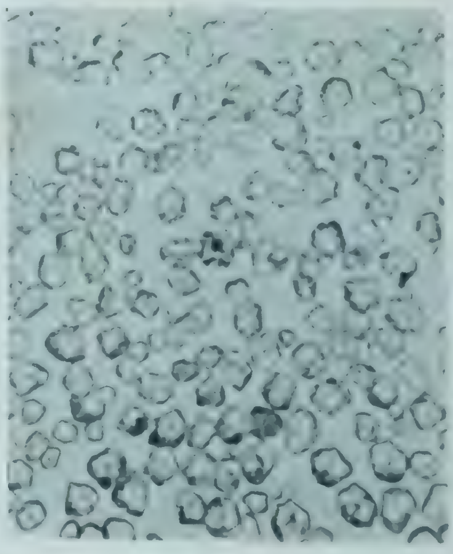


FIG. 13. Corn starch (horny); Hybrid 998, Kutias and Illinois utility.

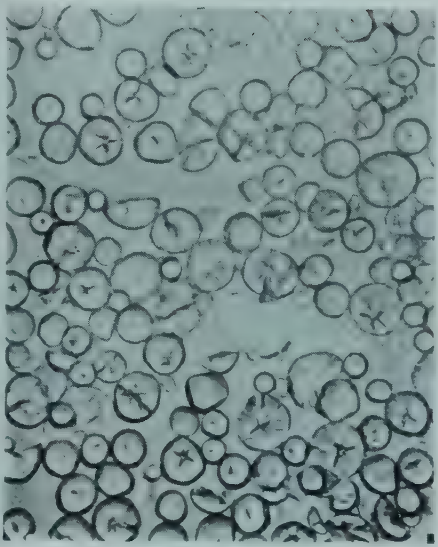


FIG. 14. Corn starch (floury); Mandan.

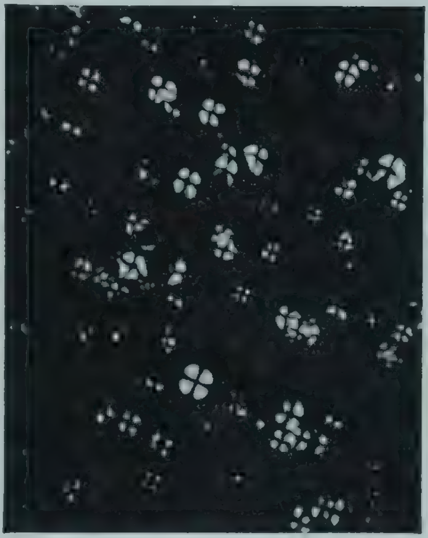


FIG. 16. Tapioca starch in polarized light.

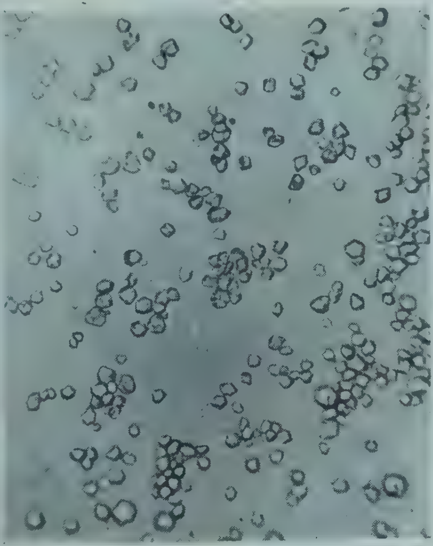


FIG. 15. Rice starch.

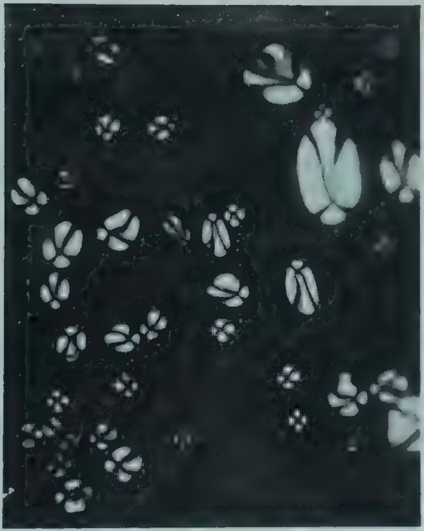


FIG. 17. Potato starch in polarized light (× 100).

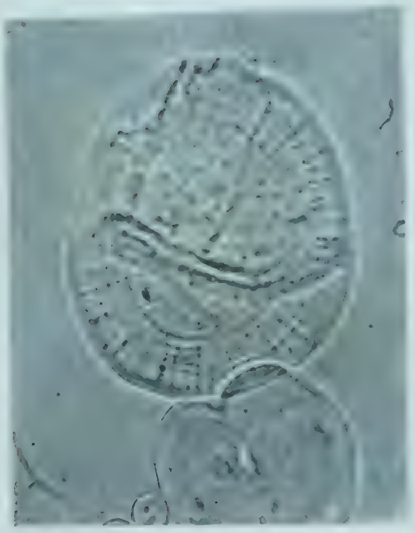


FIG. 18. Rye starch in water at 55° C (× 450).

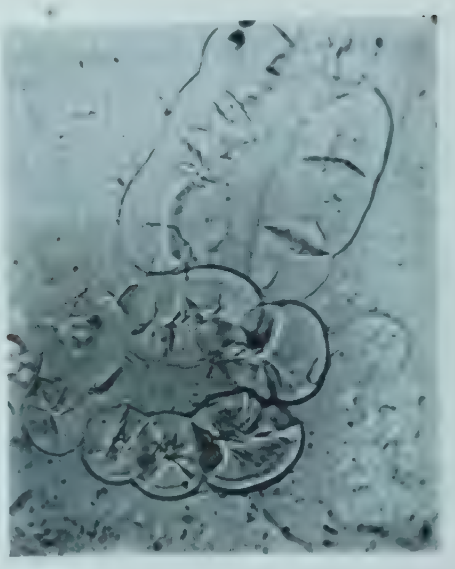


FIG. 20. Acid-modified potato starch in water at 65-70° C.

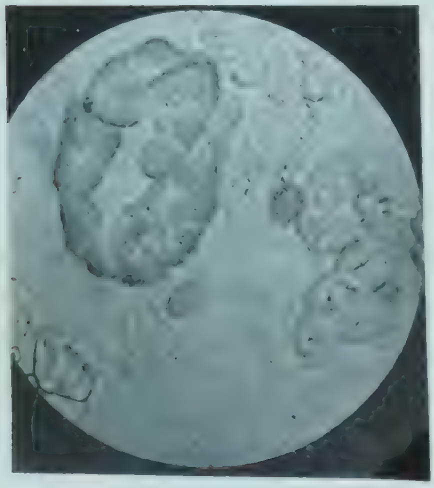


FIG. 19. Potato starch in water at 65-70° C. (× 100).

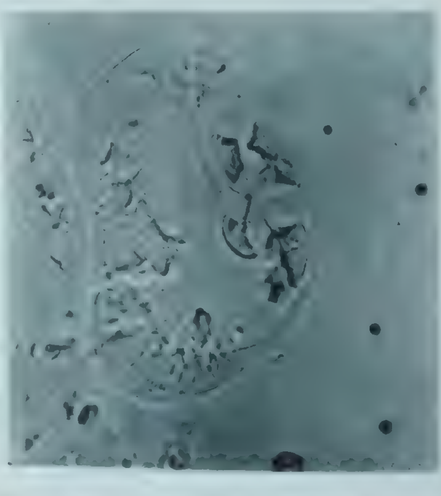


FIG. 21. Acid-modified potato starch in water at 65-70° C.

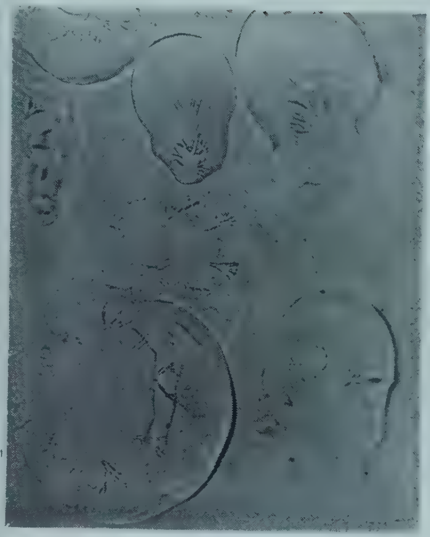


FIG. 22. Canna starch in water at 65-70° C.

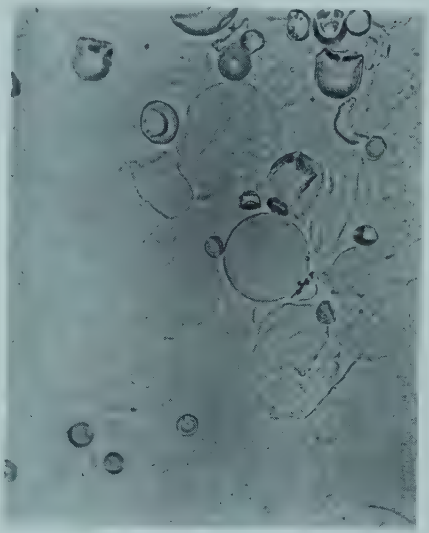
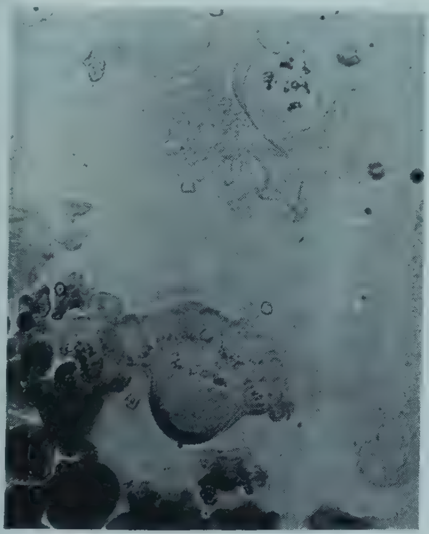


FIG. 24. Tapioca starch in water at 65-70° C.



IG. 23. Sweet potato starch in water at 65-70° C.

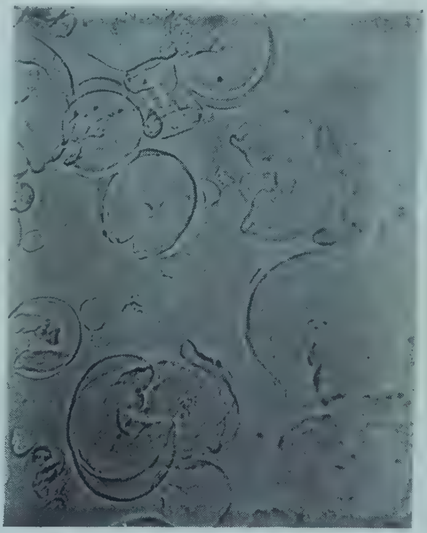


FIG. 25. Rye starch in water at 65-70° C.

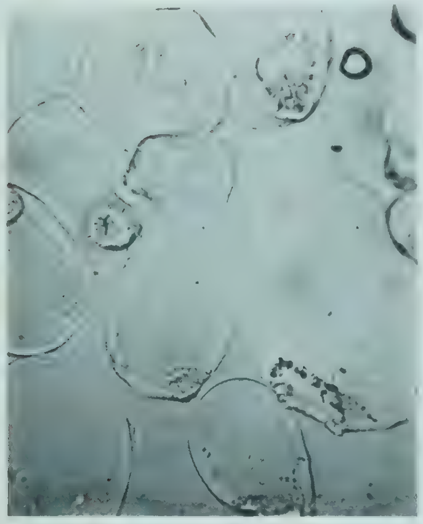


FIG. 26. Sago starch in water at 65-70°C.

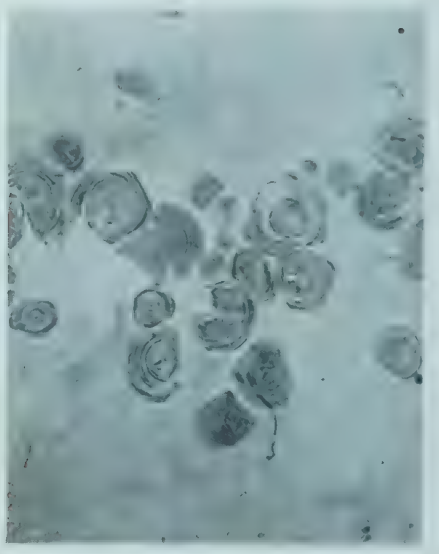


FIG. 28. Torrefaction dextrin form corn starch; early stage of solution in water-glycerol mixture.

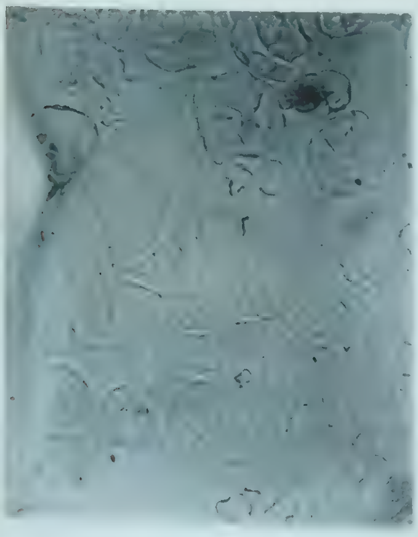


FIG. 27. Corn starch in water at 80-90°C.

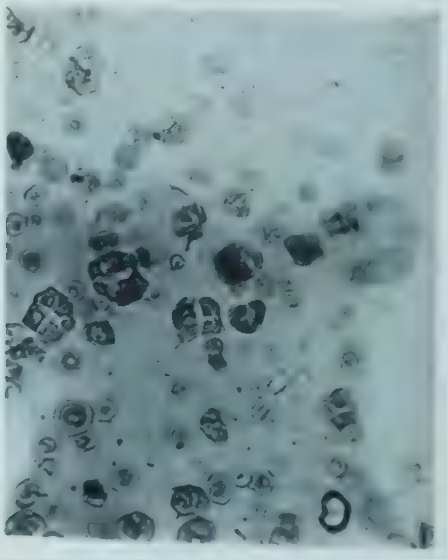


FIG. 29. Gelatinized thin boiling corn starch.

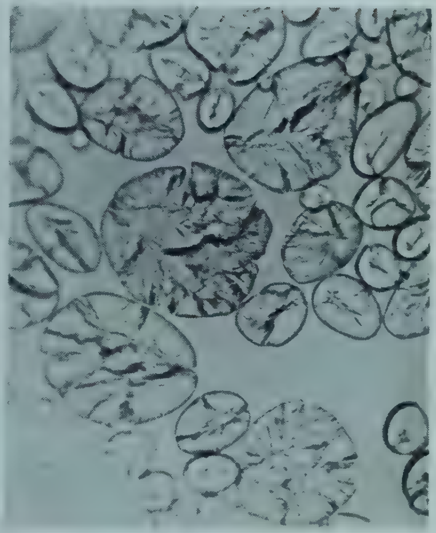


FIG. 30. Potato starch granule, crushed by pressure.

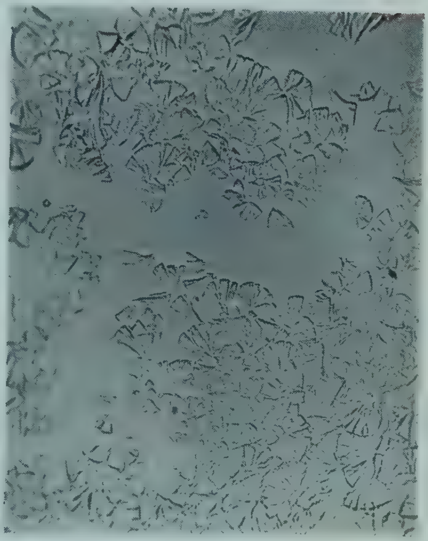


FIG. 31. Thin boiling potato starch, crushed by pressure.

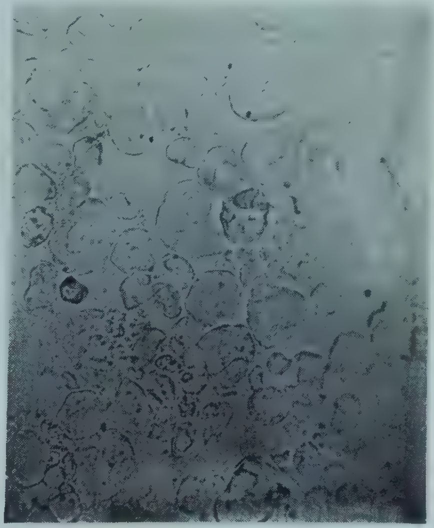


FIG. 32. Corn starch, dry ball-milled and suspended in cold water.

SECTION II. PREPARATION

CHAPTER II

THE MANUFACTURE OF CORN STARCH

Corn starch is the principal industrial starch of the western hemisphere and is manufactured in plants extending from Canada to Argentina. The United States is the largest producer. The industry was pioneered by Kingsford (1) and by Duryea and dates from about the middle of the last century. In recent years this industry has expanded in Great Britain, on the European continent (2) and in the Orient.

Corn is one of the primary grain crops of the United States, being surpassed in volume only by wheat. Nearly 3.5 billion bushels, or 210 billion pounds, of corn are produced annually in the United States. However, in contrast to wheat, most of the corn crop is grown for the feeding of livestock and remains in the region where it was grown. Only about 3% of the corn crop is milled to make starch and products derived from starch (3). About one half of this amount, or 1.5% of the crop is milled for making starch to be sold as such, the other 1.5% is used for making derived products such as sugars, sirups, and dextrins. Owing to the insignificant fractional part of the corn crop that is utilized for milling starch, little attention has been given to the cultivation of a variety of corn best suited for its manufacture. The emphasis in genetical studies has been to produce hybrids that will give the highest yield of grain per acre for particular soil and climatic conditions and that will be best suited for feeding. A soft, floury type of grain from which the starch is readily milled with relatively little physical or chemical degradation of the carbohydrate is usually less resistant to destructive biological agents than a tougher, hornier variety. The white varieties of grain, which yield a starch of brilliant whiteness, in general require a relative long season for growth and maturing, and are not favored by the farmers of the corn belt where the largest starch producing plants are located. In recent years the trend has been for farmers to plant a more horny type of grain, the characteristics of which will be compared in the following section.

The yield and quality of starch manufactured from a given weight of grain has not only been maintained but actually increased in recent years against the adverse trend in the less desirable type of grain supplied to the millers. This reflects to some extent the study and research which has been devoted to the manufacturing process.

Corn (4, 5), of which more than 500 varieties are known, is native to the Americas. It has also been introduced to and grown extensively in many sections of the Eastern Hemisphere by Europeans. In northern latitudes the seed is planted during late April or early May. The plant grows rapidly in deep, fertile, friable loam soils when the climate is warm and humid. Not only are sunshine and warm days required, but for the most rapid development, warmth during the night is also desirable. These conditions are fulfilled by the climate of the American corn belt. The plant is easily injured by drought and winds, especially when the tassels and ears (on which the grain grows) are forming. Although corn is of the family of grasses, the long blade-like leaves may be cut to shreds by arid winds such as frequently prevail on the plains west of the corn belt. The plant rises above ground in several weeks' time to a height of from 7 to 10 feet, on which a pithy, cellulose-containing cob develops. On these, some three to six to a stalk, the grain grows, protected by a sheath of leaves. Some 20 weeks after planting the grain may be sufficiently matured, and the ear is then removed from the stalk and husked. The grain may be stored locally on the cob or shelled and transported to granaries from whence it is sent to the mills.

Being a seed, corn may be stored for future use over extended periods of time if allowed to mature fully and if kept in a dry state. During maturation the moisture in the grain drops to a normal of about 20%. During storage the moisture content is reduced further, depending on the relative humidity under which it is stored. Moisture contents as low as 10% are not uncommon. On the average, however, the moisture content of grain coming to the mills a month or two after harvest is about 18 to 20%. Thereafter, until the harvest season the year following, the moisture content of the current crop arriving at the mills falls off to about 13 to 15%. As the moisture content of the grain falls below about 18%, microorganisms and enzyme systems within the grain which are capable of degrading native starch become comparatively dormant. However, as the grain dehydrates it becomes tougher and less readily rehydrated in wet milling processes. The intensity of the milling procedures, such as temperature and SO_2 concentration used in steeping and the severity of grinding, may be stepped up accordingly with the result that the starch granules may be injured, or their components slightly degraded when old, dry corn is used. Therefore, in practice, a starch of higher viscosity may be produced from new corn, or from corn which has not had an opportunity to mature fully. The writer has observed the production of starch from immature corn grain which had a paste consistency of 160 by Scott test (6), whereas starch from mature corn usually gives a value of about 90.

No significant change in the ratio of amylose to amylopectin (constituent polymers of starch) has been observed in corn starch during maturation of the grain or during normal storage. The total starch content does, however, increase during the early stages of the maturation process.

Immature corn, or grain of high moisture content for other reasons, may be dried for future use, as indicated below.

From the foregoing, it should be evident that the operation of corn starch factories is not disrupted because of seasonal changes in the availability or supply of raw material, as is normally the case for potato or tapioca starch factories.

1. **The Corn Grain.** Corn grain as now processed in the United States shows the following general analysis, at an average of 18.5% moisture content according to Bartling (7):

TABLE I

Composition of Corn Grain

	Per cent	Lbs. per bu.
Moisture	18.5	10.36
Starch	55.5	31.08
Protein	8.2	4.59
Oil	3.0*	1.68
Fiber	2.4	1.34
Ash	1.5	0.84
Pentosans	5.1	2.86
Water soluble and undetermined	5.8	3.25
	100.0	56.00

*See text.

Earle *et al.* (8) analyzed samples from 11 current varieties of corn obtained from different localities. On a dry weight basis, they found an average of 4.8% oil, with a high of 5.8 and a low of 3.9%. Analytical results of the writer shows that corn currently arriving at the mills contains 4.5% which would be 3.67% at 18.5% moisture content.

On a dry weight basis, corn grain now milled contains about 68% starch. Earle and coworkers found a low of 66 and a high of 74%.

The protein of corn consists chiefly of the relatively water insoluble zein and zeanin. They are appreciably soluble in some acids, more soluble in alkalies such as sodium and potassium hydroxides, and are dispersed in various alkaline systems. Zein is soluble in aqueous alcohol. A minor amount of other proteins, such as albumin, is also present in corn. Hansen *et al.* (9) found a linear relationship to exist between the percentage of alcohol soluble and total protein in a large number of corn grain samples.

The ash of corn grain is given by Bartling (10) in Table II.

The phytin content of corn increases as growth progresses, reaching a maximum in the seventh week when about 25 mg. of phytin phosphorus is

TABLE II

The Ash of Corn Grain

	Per cent of total ash
P_2O_5	47.60
K_2O	29.47
MgO	14.39
CaO	1.90
SiO_2	2.27
Na_2O	1.43
Fe_2O_3 and Al_2O	1.22
SO_3	0.67
Cl (in chlorides)	0.78

formed daily per ear. At full maturity phytin phosphorus amounts to 0.27%, which is 88% of the total phosphorus in corn, according to Early and DeTurk (11).

The water soluble extract of corn grain has a pH value approximately 6.0 to 6.5. About one-third of the dry substance of the extract is composed of low molecular weight carbohydrates. These may be fermented by bacteria associated with the grain when it is steeped in the warm acidulated water used in the early stages of wet milling. The chief product of fermentation at the temperatures employed is lactic acid. The acids tend to hydrate the protein of the grain and to solubilize the alkaline earth salts. The phytates may be split to phosphates and inositol; the proteins into the usual degradation products, peptides, amino acids, and NH_3.

The fat in corn is contained principally in the embryo and this will be discussed following an analysis of corn germ.

The corn grain may be considered as comprised of four regions: (a) an outer hull which encases the other three regions, (b) the embryo, or germ, which is located near the tip of the grain behind the tip cap, (c) the crown, that part of the endosperm which is light in color and is located at the opposite end from the germ (in some varieties it may extend down to and around the germ), and (d) the remainder of the endosperm which is more intensely colored than other regions, particularly in yellow varieties of corn. In addition, the hull, or pericarp, may be separated from the rest of the grain by a very thin layer of glutinous material.

The hulls contain insoluble, non-starchy carbohydrates, and inorganic constituents and are readily loosened and removed by soaking the grain in warm water followed by passage through coarse mills and then over screens.

Earle *et al.* (8) have studied the composition of the several parts of the unsteeped corn kernel. Eleven samples of corn grain were separated by hand into fractions representing the endosperm, germ, bran and tip cap. The distribution of starch, protein, oil, soluble sugars and ash in these regions is shown in Table III.

TABLE III

Distribution of Components in Corn Grain

	In endosperm, % total	In germ, % total	In bran and tip cap % total
Starch	98.0	1.4	0.6
Protein (N × 6.25)	74.8	22.4	2.8
Oil	14.5	83.7	1.8
Sugars	28.2	70.2	1.7
Ash	16.5	79.7	3.8

The germ contains most of the inorganic constituents of the grain and these are removed in the steeping process. The chief constituent of the steeped germ is the low melting point fats as is shown in Table IV. This lists the analysis for average samples obtained by Bartling (10) from a wet milling process.

TABLE IV

Composition of Steeped Corn Germ

	Per cent
Moisture	1.6
Oil	56.5
Protein	11.5
Fiber	9.0
Starch	8.0
Water Soluble	2.6
Mineral Matter	1.0
Pentosans and undetermined	9.8

Owing principally to the high oil content, the germ has a lower specific gravity than the endosperm and because of this the germs in roughly ground, steeped corn may be separated from the rest of the grist by levitation. After washing and drying the germ, most of the oil may be pressed out by the use of heated expellers, or removed by extraction with solvents.

The composition of the oil, an economically important by-product in corn starch manufacture, is shown in Table V. The oil is comprised mostly of the glycerides of the fatty acids listed. Both the analytical values of Baur and Brown (12) and of Kummerow (13) are given and these show a very good agreement. Thaler and Broseff (14) have found some differences in the analyses of germ oil obtained from Argentine and Bulgarian corn. These are attributed to climatic differences under which the grains were grown. These workers also found about 2% of linolenic acid.

TABLE V

Composition of Corn Oil

Fatty acid	Percent of total fatty acids (Baur and Brown)	(Kummerow)
Myristic	0.1	0.5
Palmitic	8.1	9.7
Stearic	2.5	3.1
Palmitoleic	1.2	0.2
Oleic	30.1	30.4
Linoleic	56.3	55.6
Above C_{18}	1.7	——

The crown region of the endosperm contains starch which is packed quite loosely in contrast to the tightly held starch embedded in the horny regions of the endosperm. Crown starch consists primarily of round granules which are readily loosened from each other by a very limited steeping period in warm water and a moderate amount of milling. Cross sections of layers of the horny regions show smaller polygonal starch granules embedded in sheets of glutinous material. The appearance is that of a cross section of concrete, the crushed rock corresponding to the starch and the cement to the gluten. Penetration of water into these regions of the grain is very slow. Indeed, loosening of the starch in the horny regions can only be accomplished with the efficiency demanded in practice by the addition of solvating agents such as acid or alkali to the steeping liquor or by enzymic action, followed by comparatively intensive grinding operations. The acidity and time of contact necessary to hydrate the horny gluten and the grinding required to expose the starch granules so that they may be removed from the sheets of gluten is sufficient to cause a material degeneration of the starch when these conditions are applied to the crown region.

Distinguishing characteristics for many varieties of corn grain are the proportion of crown to horny starch and the shape of the crown region. In the more floury types, the crown starch predominates. The starch obtained from a floury corn grain is shown in Fig. 14. Some of the floury types of grain contract in the crown region during the maturing period so as to show a very noticeable indentation. These are classed as Dent corns. The tougher varieties are classed as Smooth Dent and as Flint. An extreme example of the horny type of corn is Hybrid #998, a cross between an American Utility type (medium horny) and the Russian Kutias, which has no crown starch whatsoever. The starch obtained from Hybrid #998 is shown in Fig. 13. Corn now arriving at the North American mills is a mixture of various hybrids grown locally and these are medium horny varieties. Starch from these types are shown in Fig. 12.

Modern milling practice demands a yield of starch above 60% and values as high as 67% are commonly reported, even though the corn now milled is a relatively horny hybrid. Milling operations are, therefore, made rather severe in order to recover the major share of the horny starch within the grain. The result is that the paste viscosity of the recovered starch by Scott test (6) is about 90, whereas the floury starch which is obtained from ground corn without steeping has a Scott test of 140 (15).

Horniness is not necessarily related to the protein content of the grain. More likely, it is due to the physical state of the water insoluble proteins in the endosperm. Two other extreme types of hybrids are interesting to note at this point, an Illinois high and low oil content hybrid corn. Comparative analyses of these are given in Table VI.

TABLE VI

Comparative Analyses of High-and Low-Oil-Content Corn

	Starch, %	Protein, %	Oil, %	Ash, %	Test weight, lbs./bu.
Illinois Low Oil Hybrid	69.57	12.29	1.24	1.39	53.0
Illinois High Oil Hybrid	54.62	13.74	12.23	1.83	58.5

Test weight, or specific gravity, at a given moisture content may be taken as a measurement of horniness. It will be observed that the high-oil corn is the more horny. Even though it contains ten times as much oil, its test weight per bushel is considerably higher than the low-oil hybrid. Inasmuch as the protein contents are not significantly different, and the high-oil corn contains about 15% less starch, it may be concluded that the more horny nature of the latter is due to a more organized packing of the protein layers which encase the starch.

Cox *et al.* (16) have compared the analyses for the horny and floury regions of the endosperm of Reid Yellow Dent Corn. Their results are given in Table VII.

Although the protein content of the horny region is nearly twice that of the floury, nevertheless the latter does contain a rather substantial amount of protein. It may also be noted that the oil content of the horny region is more than twice that of the floury. The protein network of the horny region which appears as strands after cross-sectioning is birefringent, indicating

TABLE VII

Analysis of Horny and Floury Regions of Dent Corn

Constituent	Horny endosperm, %	Floury endosperm, %
Protein (N × 6.25)	13.25	7.69
Alcohol soluble N	1.49	0.64
Starch	80.40	85.60
Sugar	0.47	0.52
Oil (petroleum ether extract)	0.73	0.33
Ash	0.27	0.35

that molecules in the network are oriented. These workers have suggested that powerful binding forces are operative in these crystalline regions which resist solvation and account for the toughness of the protein network.

Because the wet-milling industry requires a substantial portion of the corn grain which is placed on the market, it frequently becomes necessary to mill the lower grades of corn.* Damp corn may be mixed with better grades and milled immediately or it may be dried, either at the corn elevators or at the mills. If damp corn is held in storage biochemical activity soon develops to the point where the temperature of the grain increases. If the action is not checked, as by aeration or "turning over," the grain may become so hot as to be rendered worthless for milling. As a precaution, many mills equip their storage tanks with thermo-recorders in order to be informed when, and if, the initial phases of biochemical activity have started.

Milner (17) has pointed out that two processes cause heating of grains, respiration of the grain itself, and mold growth. Respiration of the grain assumes importance in this respect only when the relative humidity and the moisture content of the grain are low. Semeniuk and Gilman (18) have made an extensive study of the storage of corn grain and the changes which take place during storage. These workers have pointed out that the water relations of corn, both the moisture content of the grain and the relative humidity of the air in which it is stored, are the principal factors in safe storage. The hygroscopicity of the corn contributes to the importance of the relative humidity and is in turn affected by the type of kernel. An inspection of bins of spoiled corn disclosed the presence of many fungi, among which *Penicillium*, *Aspergillus*, and *Rhizopus* predominated. Nagel and Semenuik (19) have further examined the growth of pure cultures of many fungi isolated from spoiled corn and have studied the chemical changes, such as carbohydrate utilization and the production of acids, produced by these organisms both on grain and synthetic media.

---

*For grain standards see "Handbook of Official Grain Standards of the United States" published by U. S. Bureau of Agricultural Economics.

The deterioration of grain that results from the activity of microorganisms usually starts from a fungal growth, the mycelia of which readily penetrate the kernel near the germ, digest the contents of the latter, and then invade the endosperm. Characteristically, damaged corn has a below normal oil content.

On the assumption that more or less undesirable biological activity has existed in low grade, damaged corn, a test has been proposed to differentiate grades by differences in the titratable acidity of the whole kernel (20). Ten grams of ground corn is titrated in a water suspension with 0.01 N NaOH and the result expressed in milliliters. Number 1, sound corn tests approximately 10 ml. whereas very low grade corn may test as high as 50 ml. At least part of the acidity is due to fatty acids which arise from the hydrolysis of the fats in the germ. Bartling (20) finds that the oil expressed from the germ of moldy corn contains more free fatty acids than the oil from sound germs.

When immature, moldy, or wet corn is dried for transit or storage before wet milling, certain changes are induced in the gluten which complicate the separation processes. Cox *et al.* (16) found that when corn samples are dried at 180 to 200°F., the protein of the endosperm (the network holding the starch particles) did not swell as much as corn not artificially dried when both corn samples were soaked in dilute sulfurous acid 24 hours at 49°C. In addition, the starch was held more tenaciously by the protein matrix in the artificially dried sample and although protein dispersal appeared to be normal during the soaking period, the disintegrating matrix left pieces which adhered closely to the starch granules. Another sample of corn, artificially dried at 120°F., appeared intermediate in behavior between corn dried in the field and that dried at 180 to 200°F. Low grade corn which has been treated at the grain elevators by procedures involving kiln drying, and without due regard to the principles disclosed above, is an undesirable raw material for the miller.

2. Cleaning and Steeping. The corn grain is delivered to the miller by railroad in box cars. In many mills the grain is unloaded by power shovels although in some mills the car is run onto a platform which tilts it to empty by gravity flow. The grain falls into a pit from whence it is carried by screw conveyor to a bucket elevator which raises it to the top of a cleaning or winnowing mill. Here, by air currents, the dust, chaff, broken grain particles and other light material are removed and collected. Screens are used to remove stray cobs and other extraneous material and magnetic devices may

be used to remove metallic objects which may have fallen into the grain in transit.

Normally, the corn is transported to concrete bins which may hold as many as 40 carloads each. The usual custom is to maintain about a thirty-day supply of grain although occasionally the supply held in storage is less. If wet grain is received and drying facilities are inadequate it is generally milled directly.

From the large storage bins the grain is transported by elevator and by continuous belt conveyor to a weighing device where the grain is tripped into hopper bins which supply the first units of the wet milling system. Wet milling is the common practice, a brief outline of which is shown in Fig. 33. For this purpose, the corn is first soaked in warm water for various periods of time and under other conditions which are varied with the nature of the corn. This procedure is called steeping.

Several objects are to be achieved in the steeping process, the most important of which are as follows:

1. To soften the kernel so that the various components of the grain, such as the hull and fiber, the germ (which contains nearly all of the oil), the gluten, and the starch, may be separated from each other with a high degree of efficiency.
2. To remove a portion, at least, of grain components that are soluble in warm water.
3. To reduce or inhibit the activity of microorganisms that are brought into the mills on the grain.
4. To complete the cleansing of the grain.

The softening action is by far the most important and complex; important because the efficiency of the milling operations in respect to the recovery of components depends upon it and complex because of the difference in chemical composition of the various grain constituents or parts which are to be conditioned. Hydration is an important factor of the softening action and the water content of the corn increases rapidly at first to about 35-40% and then levels off at about 43-45%. At this level, the hulls and germs are readily removed by a coarse grinding operation. However, even at a satisfactory moisture level the starch cannot necessarily be loosened from the gluten in which it is embedded and it is apparent that (a) either some change in the physical nature of the protein is required or (b) the solvation of a cementing substance holding the granules in the protein is necessitated. Existing information, given below, favors the former view, that the protein itself is the cementing agent. The germs are separated first from the coarse grist by flotation. This is made possible by the lower specific gravity of this component which is due to its oil content, the water which it takes up during steeping, and by the removal of a portion of the water soluble solids in the steeps.

After removal of the hulls by passage over screens and a fine grinding to loosen the starch from the gluten the latter two are separated from each other by sedimenting the starch and floating the gluten. Even though the starch has been loosened from the protein network of the kernel, efficient sedimentation of the starch can be accomplished only if the steeping operation has been properly performed. The physical state of the gluten is important as is also the presence of a sufficient quantity of electrolytes, which should not be unduly removed by leaching in the steeps.

Various agents have been suggested and used to limit microbiological activity. However, SO_2 is now almost universally employed. This is due to its low cost, its pronounced germicidal properties under controlled conditions and because it may contribute to other favorable actions which are desirable in steeping. Lactic acid bacteria are the type most commonly found in steeping media. Some yeast-like organisms are present which may develop at the lower temperatures used in subsequent milling and separation procedures, and fungal growth usually ensues where moisture and temperature are suitable and if a sufficient concentration of SO_2, or other germicide, is not maintained. Thus, if microbiological activity is permitted, the fermentable carbohydrate, such as the soluble sugars, may be converted to lactic acid, acetic acid, alcohol, and other products. Some of the organisms can hydrolyze, or convert, accessible protein into its degradation products. A part of this biochemical activity is desirable. Native protein that dissolves or disperses in the steepwater coagulates or denatures to form an insulating coat on the heating elements of evaporators used to concentrate and recover the soluble corn solids after steeping. A partial degradation to a non-coagulable stage is therefore desirable. However, if this activity is uncontrolled or too rapid, large amounts of native protein will be dissolved, maintaining a saturated solution in respect to the protein. Meanwhile, the dissolved protein is being split into low molecular weight bodies. The degradation of the protein will proceed into the last stages and amino acids and nitrogenous bases may permeate the starch and very materially increase the difficulty of obtaining a pure starch in the final washing stages of the wet milling process. Adsorbed nitrogenous material on starch is undesirable when the starch is converted to sugars and sirups in manufacturing units following the milling process. These materials tend to change, unpredictably, the acid activity used in the hydrolysis and favor the creation of colored bodies which lower the quality of the end products. Some biochemical activity is desirable in respect to lactic acid production. Lactic acid tends to soften the gluten in the steeping process (16) and, being nonvolatile, it is retained by the steepwater during evaporation after steeping, thereby tending to hold magnesium and calcium salts, also present in the water, in solution as acid salts. Otherwise, these salts form insoluble crusts, or scale on the heating units of the evaporators. However, the creation of large amounts of lactic acid is undesirable for several reasons: The solvation of native protein is promoted to an undesirable extent and the lactate ion has an adverse effect on the sedimentation of starch from finely ground mixtures of starch and gluten.

The presence of soluble phosphates, formed in part from phytin, is desirable since these electrolytes increase the sedimentation rate of starch in the presence of finely ground, hydrated gluten.

Practical operators in the mills sum these considerations by stating that an object of steeping is an efficient removal of substances in corn originally soluble in water without the creation of undesirable new soluble material.

Killinger (21) has discussed the control and use of SO_2 in respect to its germicidal properties in wet milling. Free SO_2, or sulfurous acid, is an effective inhibiting agent for the organisms studied at relatively low concentrations (0.02 to 0.03%) and its efficiency is markedly increased by raising the temperature over the range from about 30 to 50° C. However, a considerable portion of the SO_2 added to process water does not remain free; it reacts to form bisulfite and to form addition compounds with amino groups, ketoses (22) and with carbohydrates, possibly through the aldehyde group, and these combined forms are very much less effective sterilizing agents. A determination of SO_2 in process water by titration with standard iodine solution, a procedure formerly used in the industry, conveys little information on the relative germicidal properties of the SO_2 present. The determination of free SO_2 by extraction with benzine is recommended. The efficiency of the germicide is reported to be not necessarily attributed to the acidity (pH) required to maintain the SO_2 in the "free" state.

The function of SO_2 as a steeping agent has been investigated by Cox *et al.* (16). These investigators showed that starch is embedded in and tightly held by a protein network which is birefringent. During steeping the network swells and tends to form tiny globules of hydrated protein. With time, the protein loses its birefringence, tends to disperse and finally the undispersed portion is so weak that after removal of the starch granules it collapses against the cell walls and shows little evidence of the original network of films. SO_2 greatly accelerates this process. It was found impossible to steep corn satisfactorily by soaking the grain in distilled water at 49° C. for 24 hours and after milling only 64% starch recovery was obtained. SO_2 accelerated the steeping action in proportion to the amount added as is indicated in Table VIII. However, the addition of acetic acid, added in an amount equivalent to 0.2% SO_2, did not give comparable results; the corn was hard to grind and separations were difficult. Poorer results were obtained with HCl. On the other hand steeping with 0.2% lactic acid produced protein disintegration about equal to that given by a 0.1% SO_2 steep and a softening action approximating that produced by a 0.2% SO_2 steep. The addition of lactic acid to SO_2 steeps increased the softening action of the latter. These workers attribute the specific action of SO_2 to its reducing properties rather than to its acidity, inasmuch as sulfites exert a dispersing effect on some proteins and reducing agents activate papainases (23) which may also play a part in the general action.

The principal objections to the use of SO_2 arises from the fact that at the temperature and pH required to secure its beneficial effects, the SO_2 volatilizes from aqueous solutions and that these solutions are acid. Its corrosive action is high not only on equipment but also on structural parts of process buildings. The acid tends to modify the starch by a hydrolytic action, which is first noted by a significant loss in potential paste viscosity of the starch.

TABLE VIII

Effect of Steeping Adjuncts on Corn at 49°C.

Steeping medium	Steeping time	Condition of protein matrix	Yield of starch %
Distilled H_2O	4	Coarse, holding starch tightly	—
Distilled H_2O	24	Slightly less coarse, but holding starch tightly	64
0.1% SO_2	4	Slightly less coarse than unsteeped corn but holding starch tightly	—
0.1% SO_2	24	Somewhat less coarse but holding starch tightly	82
0.2% SO_2	8	More dispersed than after 24 hrs. steep in 0.1% SO_2	—
0.2% SO_2	24	Not appreciably different from 8 hrs.	83
0.3% SO_2	4	Much dispersed	—
0.3% SO_2	24	In separate particles, no network remaining	88
0.4% SO_2	4	Indistinguishable from 4 hr. steep in 0.3% SO_2	—
0.4% SO_2	24	Indistinguishable from 4 hr. sample	89
*Acetic acid	24	Hard to grind, poor separation of starch	70
*HCl	24	Easy to grind, but poor yield due to large gluten particles	56
0.1% Lactic acid	24	Softening about equal to a 0.1% SO_2 steep	—
0.2% Lactic acid	24	Softening about equal to a 0.2% SO_2 steep; protein disintegration about equal to 0.1% SO_2	—

*Equivalent concentration to 0.2% SO_2.

*: pH of the medium?

Accordingly, considerable attention has been given to a study of other steeping agents, particularly those less acidic than SO_2. Sodium benzoate has been proposed (24) but apparently the addition of some SO_2 in this system is required as well. The use of organic halogen compounds such as monochlorobenzene and *o*-chlorotoluene have been patented by Berquist (25). Bartman (26) claimed the use of formaldehyde to be effective and Eckland (27) recommended the use of ozone.

Stratton (28) was among the first to advocate the use of an alkaline steeping medium and the alkaline process described by Kingsford (29) was used for many years until it was abandoned for the more efficient SO_2 process. More recently, Wagner (30, 31) has again directed attention to the use of caustic alkalies.

Other workers have experimented with steeping technique in order to employ SO_2 to best advantage, that is, to obtain the benefits of this specific steeping agent and to minimize the disadvantages, as noted above. Some of these results will be mentioned in a discussion of steeping methods which follows.

The most elementary method of steeping consists of partially filling a wooden vat, holding about 2000 to 2500 bushels of corn, and covering the grain with warm water containing a steeping agent such as SO_2. The temperature is maintained at about 50°C. for 40 to 48 hours after which the steep liquor is drained from the softened grain which is then sent to the mills.

The principal objection to this method is the relatively large amount of water which is required to soften the grain and to remove a sufficient proportion of water soluble constituents. Modern milling and refining systems are designed to handle steeped grain containing between 3 and 4% water soluble material; some require a lower percentage.

This system does, however, possess certain inherent advantages when comparison is made with systems employing countercurrent, or diffusion cell principles, designed to obtain a more efficient removal of soluble material for the use of a given quantity of steeping liquor. As pointed out by Kerr (32), the single vat system readily permits the addition of germicide at the place where it will be most effective. Both the incoming corn and the process water from the mill house, which is the liquor usually used for steeping, are the principal carriers of microorganisms into the steeps. Therefore, since both the grain and the process water are exposed to the highest concentration of SO_2 used in this system, it follows that, in order to limit microbiological activity to a given level, a smaller addition of germicide is required here than in countercurrent systems wherein the incoming grain is exposed to the lowest SO_2 concentration.

If SO_2 or other hydrolytic agents are used in steeping, then the corn in single vat steeping is subject to lower concentration of acid and consequently the starch may be exposed to less degradative influences. Moreover, the acid activity or H-ion concentration in steeping liquor is not necessarily the same as the pH of the water phase within the grains or the moisture layers adjacent to the starch granules. The H-ion concentration of acids which enter the grain is reduced by the natural buffering systems of the corn such as phosphates and phytin. These buffers are soluble in warm acidulated water and if an excessive proportion of steeping liquor is used, particularly by a system using a countercurrent flow principle, the buffers are soon removed. The grain then gradually assumes the pH of the steeping medium. It can be seen that in single vat steeping, conditions are favorable for maintaining a large differential between the pH of the corn and the pH of the steeping medium.

The result of the advantages noted is that starch obtained from corn steeped by the single vat method has a relatively high paste viscosity. Because of the poor economy in the use of water in the single vat system, steeping is now done by methods which employ countercurrent flow. Indeed, the entire mill house, in addition, operates on this principle as well. Fresh water is used in the last stages of washing the refined starch. This water is then reused in a preceding phase of starch manufacture, such as flushing the starch from the sedimentation tables. Finally, after having been used in turn in the various operations in the mill house and having attained a content of water soluble material between 400 and 1000 grains per gallon, the water is run over an absorption tower where the desired amount of SO_2 is taken up and it is then sent to the steeps.

One of the most common forms of countercurrent steeping is the use of about 12 to 14 wooden vats, or open steeps as they are called in practice. These are, in effect, arranged in series. At one end of this series, one steep is filled with dry corn. At the other end, one steep contains corn which has been fully steeped, and this is emptied by running the steeped grain to the mills. To the next steep from this end of the series, that is, the steep which will be the next to supply the mills with corn, fresh steeping liquor is added. When this steep is full, the water may be circulated by pump from the bottom of the steep through a heating device to maintain the temperature at the desired level and then to the top of the same steep. After a predetermined length of time, the water is removed from all steeps in turn, and advanced to the next steep in the direction of the one which was last filled with dry corn. After the water has been used on dry corn the liquor is then pumped to evaporators to recover the soluble solids.

Obviously, the advancement or change of water in the series of steeps starts by taking water from the steep most recently filled with corn and water, discarding a part of the water as spent steepwater, and using the balance to cover partially the corn in a steep into which dry grain has just been placed. Water is then advanced to cover the corn in the steep from whence the spent steepwater was taken and also the steep into which the dry grain was placed. Usually two or more advances of water throughout the system are required in order to fill the last two steeps mentioned. This is because of the removal of water as spent steepwater and because of a depletion of the liquid phase by absorption into the grain particularly in the early stages of steeping. The very large amount of manual labor and supervision required can readily be appreciated. For this reason, it often becomes customary to by-pass steeps when water is advanced in order more conveniently to cover the dry corn with sufficient liquor. In this case, full use is not made of all the steeping liquor passing through the system. Another disadvantage of this system is that, since the pumping of steeping liquor from one steep to the next is discontinuous, the grain remains uncovered for a substantial portion of the steeping period. When a volatile steeping agent, such as SO_2 is used, one of the serious disadvantages of this system is the large loss of steeping agent which occurs during the many pumping operations. Not only are costs increased because of the loss, but also the volatile acid is corrosive to equipment and a physiological irritant to the workers, so that the installation and maintenance of a ventilating system is required.

Some of the mechanical disadvantages of the use of open steeps in series are avoided by employing closed iron steeps, each connected to the next by pipe from the bottom of one to the top of an adjacent steep. Steeping liquor is applied at one end of this system under a hydrostatic head sufficient to cause it to flow through the entire series and void itself at the other end as spent steepwater. Discontinuity of steeping is only momentary and this is when a steep filled with dry grain is connected into the system and when one is disconnected at the other end, drained of steeping liquor and its contents sent to the mills. Full advantage is taken of countercurrent flow in the extraction of soluble material.

The results achieved in respect to the separation of starch and its quality are, however, approximately the same for the two systems described. In both there is a measurable loss of potential paste viscosity which is attributed to the removal and neutralization of buffers within the grain and a rapid drop of the pH to a value approximating that of the steeping medium (32). When 10 to 11 gallons of steeping liquor, containing 0.15 to 0.20% SO_2 and an acidity of pH 3.5 to 3.8, are added per bushel of corn, and the steeps maintained at 125° F. for 40 to 48 hours, the pH of the grain will be reduced from about 6.4 to about 4.0 and the starch obtained will have a paste viscosity of about 90 by Scott test. Using corn of normal moisture content, about 5 or 6 gallons of steepwater per bushel of corn is obtained which contains about 6% of the weight of the grain as soluble and suspended material, has a pH of 4.0 to 4.2, and contains about 0.04% SO_2. The steeped corn grain contains about 3 to 3.5% water soluble material and approximately 45% moisture. The use of high moisture corn necessitates either the addition of less steeping liquor or the withdrawal of more steepwater, per dry unit weight of corn. The latter procedure is usually employed. Poor separations in milling usually result for several reasons, one of which is an excessive removal from the corn of ionic substances which facilitate the sedimentation of starch from gluten. Considerable thought should be given to adjusting the time and temperature of steeping, and the volume and SO_2 content of the steeping liquor to the values which are most suitable with respect to the physical characteristics of the grain to be processed.

The paste consistency of starch within untreated corn grain is probably equal to about 125 by Scott test, in some instances higher. Starch obtained from corn which has been steeped by the use of the single vat system has a paste consistency of about 115 by Scott test when a steeping liquor of the same characteristics as described above for countercurrent flow is used and a temperature of 125° F. is maintained for 44 hours. As high as 120 Scott test may be obtained if the steeping liquor used contains 0.08 to 0.10% SO_2, which value appears to be ample for this process.

Several variations in circulating the water or in applying the germicide have been tried in order to retain the principles of countercurrent flow and to eliminate the disadvantages mentioned. In one arrangement, the steeping liquor, after leaving the sulfur tower, is first run over a steep of dry corn and then applied to the end of the series which contains corn steeped the longest time. Three advantages are apparent. The temperature of corn entering the system is raised more quickly; better use is made of the SO_2 employed to inactivate organisms both in the process water used and on the incoming corn; the high peak of SO_2 concentration prevailing in the early stages of countercurrent flow steeping is reduced. In another modification, SO_2 is introduced at several places in the series of steeps. The SO_2 content of the steeping liquor is kept within narrow limits. However, in this procedure, either several additional sulfur towers are required, one for each location where, between the steeps, the SO_2 content of the liquor is to be raised, or else if H_2SO_3 is mixed into the steeping liquor at these several places, the water consumption required for steeping is increased owing to the low strength of acid made by the absorption method.

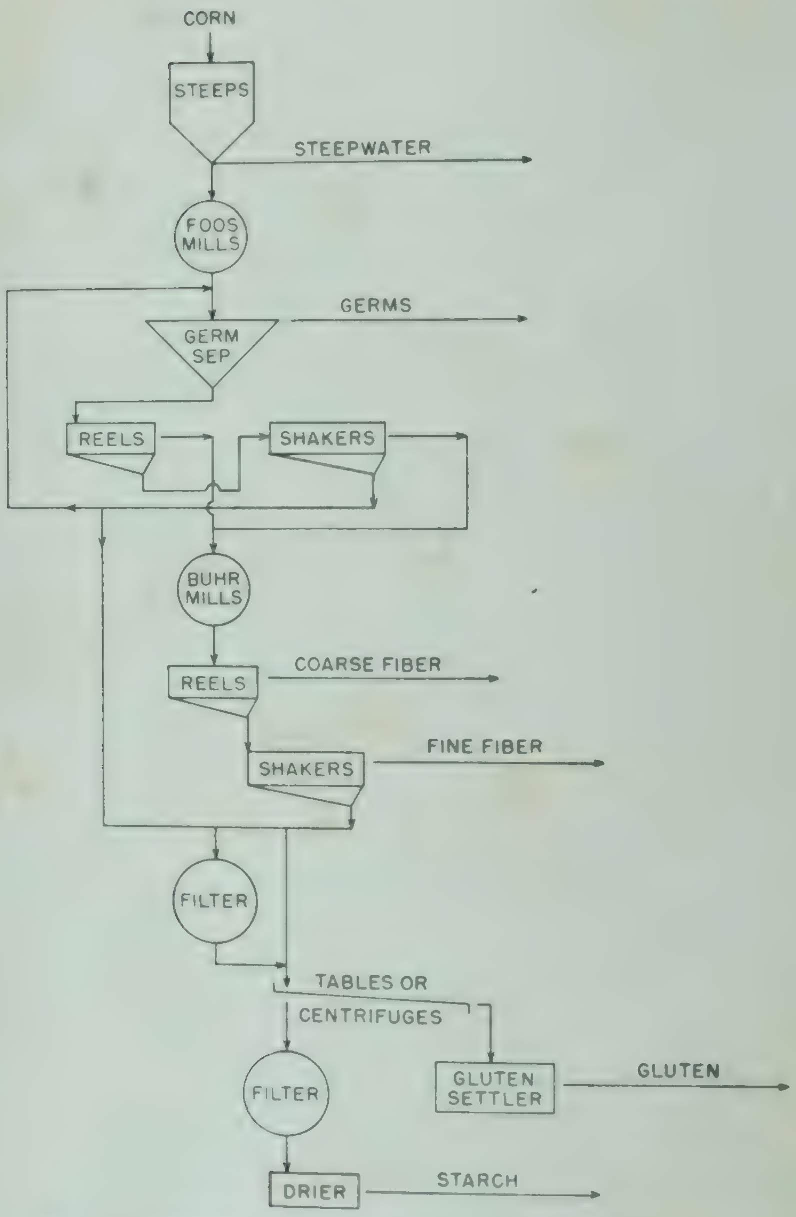

CORN
STEEPS
STEEPWATER
FOOS MILLS
GERMS
GERM SEP
REELS
SHAKERS
BUHR MILLS
REELS
COARSE FIBER
SHAKERS
FINE FIBER
FILTER
TABLES OR
CENTRIFUGES
FILTER
GLUTEN SETTLER
GLUTEN
DRIER
STARCH

FIG. 33. Diagrammatic outline of wet milling process for corn.

Another arrangement has been proposed by Kerr (32) wherein only three steeps are used in series and the time allotted to the third and last steep is short, about 1 to 2 hours. In this last steep, the corn is treated with what amounts to a wash, using gluten overflow water made 0.08% SO_2 and the corn is then sent to the mills. All of the water from this steep is placed on corn in the second steep for a period of 22 hours. A portion of the water from the second steep is discarded (about 0.8 gallons per bushel) and the balance is sent to the first into which dry corn has been placed. Gluten overflow water from the sulfur tower is used to fill the steep and to raise the SO_2 content of the steeping liquor to 0.12%. All of the liquor from this steep is discarded. This amounts to about 5.5 gallons per bushel of corn of normal moisture content and with the 0.8 gallons discarded from the second steep, a total of about 6.3 gallons of steepwater is obtained. Microbiological activity is very well controlled, for a minimal addition of SO_2. Steeped corn is obtained containing about 3.75% water soluble material. The pH of the grain is maintained above 4.5 up to the last washing stage. Starch with a paste viscosity of about 110 by Scott test is obtained after milling the corn.

3. **Milling and Separation.** After steeping, the corn is passed through several grinding and sieving operations during which the germs, hulls, fiber, and other constituents are progressively removed and finally only starch and gluten remain. (Fig. 33).

The corn is passed first through coarse mills. These are frequently called degerminating mills since the primary object at this stage is to crack open the grain and free the germ, preferably without breaking or crushing the latter. Other objects are to loosen the hull and coarsely disintegrate the endosperm. Equipment suitable for this purpose is the Foos mill which consists of two metal plates, 36 inches in diameter and studded with metal teeth. The plates stand upright in a bronze-lined housing. One plate is stationary while the other revolves at about 900 r.p.m. Figures 34 and 35 show Foos mills.

Process water is added to corn entering the degerminators and additional process water containing starch in suspension is added to the macerated corn in order to form a slurry which can be pumped to the germ separator vats and in order to adjust the density of the slurry to a value at which the germs will float. This value is about 8 to 10.5° Bé. The temperature is 90 to 95°F.

Germ separators are V-shaped vessels about 4 feet wide, 6 feet high, and 10 to 15 feet long. The magma enters at the top at one end. The hulls and coarse grits tend to settle out as the slurry is propelled to the opposite end of the vat by means of an agitator near the bottom and this heavier material is removed through an opening in the bottom of the vat. The germs tend to float and are taken off the top by overflowing into a weir box at the end of the vat opposite to that at which they entered. Usually a series of paddle wheels or drag sweep paddles aid the movement of the germs across the surface. See Figure 36.

The finer particles and liquor are removed from the heavy material leaving the germ separators by passage over copper sieves of the rotating type which are generally referred to as reels. The advantage of rotating reels over

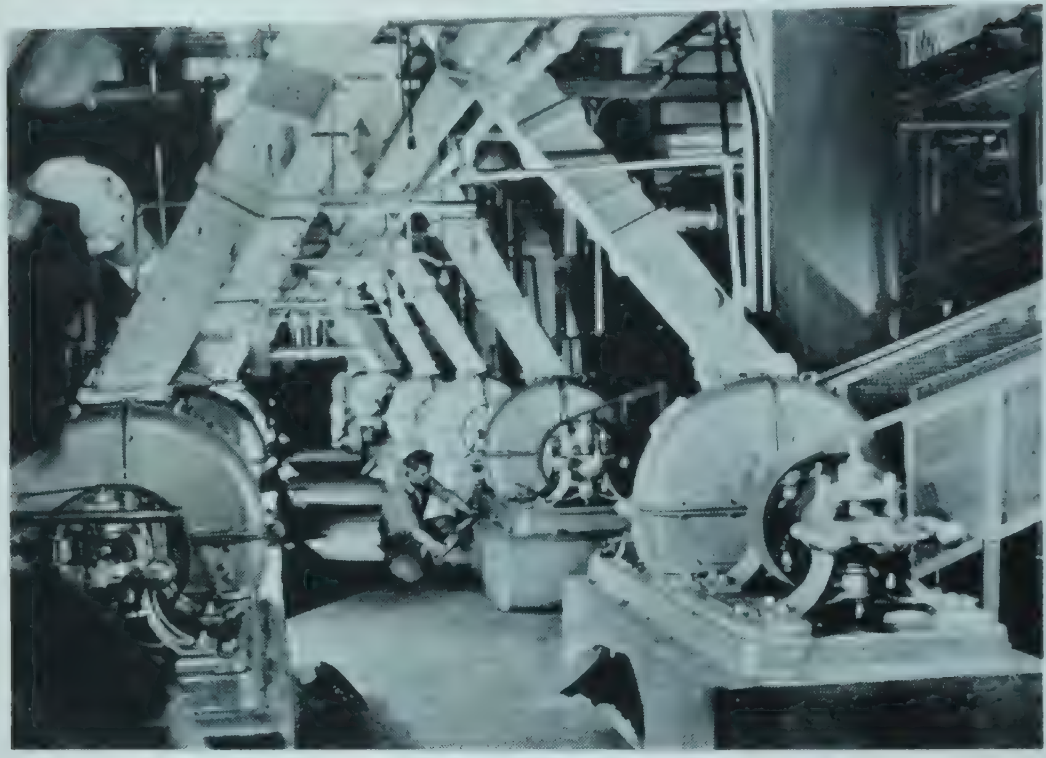


FIG. 34. Foos mills.

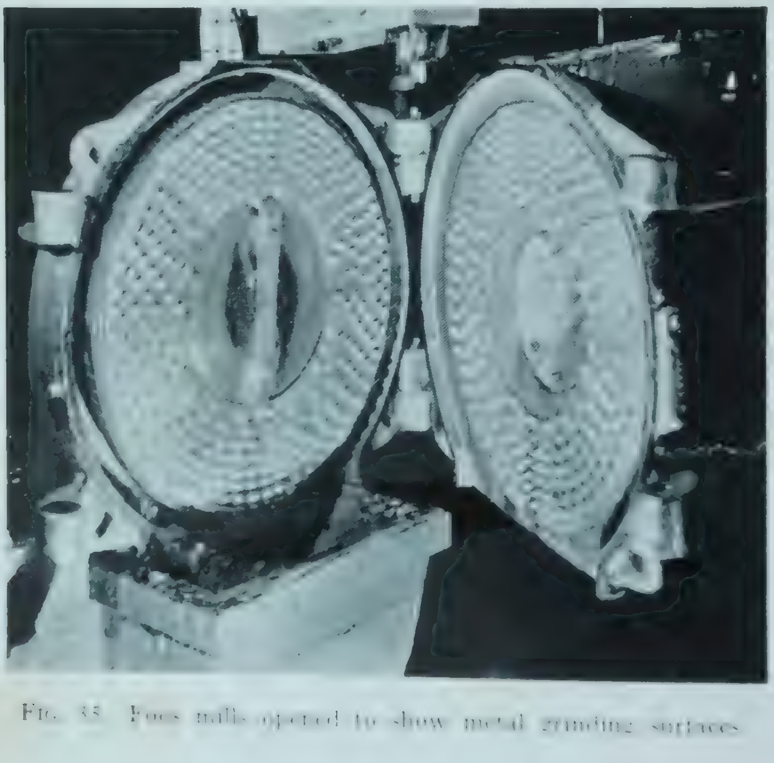


FIG. 35. Foos mills opened to show metal grinding surfaces.

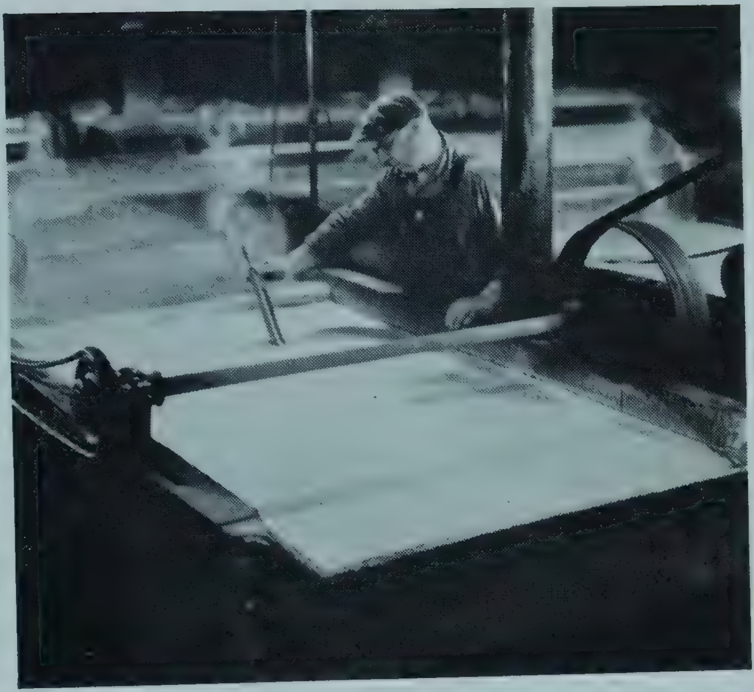


FIG. 36. Germ separators.

shaker sieves for separations of this type are pointed out in the patent to Jeffries (33). The coarse material that contains the hulls and a small percentage of germs and coarse grits is passed to a second set of degerminating mills. The grist is combined with the liquors from the reels and, after adjustment to a density of 8 to 10° Bé., the magma is passed to a second set of germ separators.

The germs are collected and washed on a series of reels to remove the adhering starch. The germs are dewatered in squeeze presses and driers and are then treated either in heated expellers or solvent extraction systems to remove the oil they contain. Reels are shown in Figure 37.

The degerminated underflow from the separators is passed over a series of reels to recover the liquor and very fine material which, after passage over silk sieves, may be returned, in part, into another cycle of the germ separating process, or it may pass on to the system supplying the starch tables or centrifuges. The coarse grist which contains hulls, fiber, and particles of the endosperm are finely ground in stone mills. These are usually buhrstone mills and consist of a furrowed grinding stone, revolving at high speed above a similar stationary stone. The object is to produce a high degree of disintegration in order to free the starch granules so that they may now be separated from the hulls and fiber in the sieving and washing operations

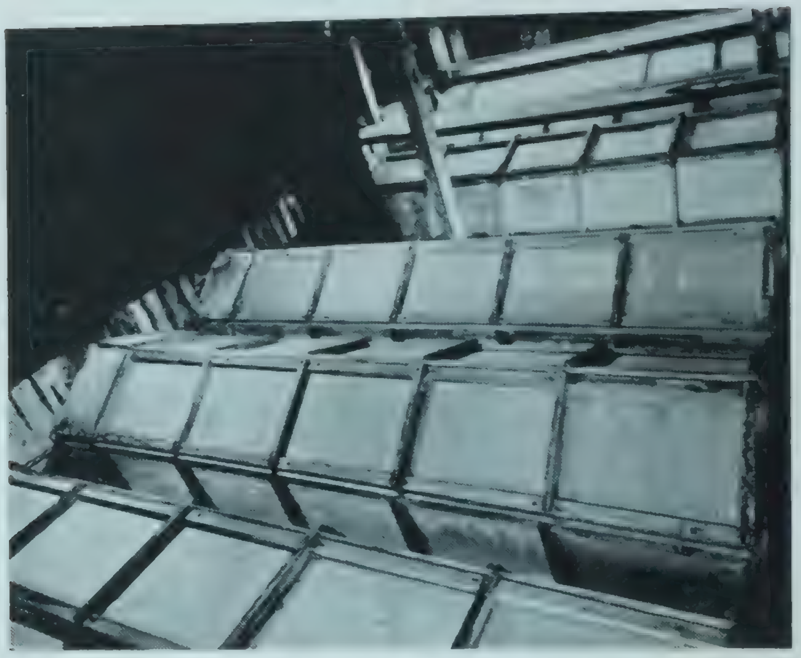


FIG. 37. Battery of reels.

which follow. In this process, the glutinous portion of the endosperm becomes very finely divided as well, so that, when the hulls and fiber are removed by passage over reels and shakers, a slurry of starch and gluten remains. The coarse material from the buhr mills is removed and washed on a series of reels after which the fine fibrous material is removed on silk shaker sieves. The presence of relatively small amounts of fiber interferes with the separation of the starch from gluten in the sedimenting or centrifuging operations. Shakers are shown in Figure 38, buhr mills in Figures 39-40.

The slurry of starch and gluten is now adjusted to the desired density for separation of the starch. Even though a countercurrent principle is used in washing the adhering starch from the various grists, the recovered solids usually do not increase the density of the liquors to the value desired in some systems for tabling or centrifuging the starch. In such cases, the liquors are partially dewatered by passage over Feinc or string filters. These are continuous, revolving drum, suction filters resembling a large Oliver filter but equipped with a series of strings which pass around the drum and thence to a roller at some distance so that the layer of filter cake is readily lifted from the drum by the strings.

The most elementary method for separating starch from dispersed gluten is to run the mill liquor into a vat, allow the starch to settle for several hours and then draw off the supernatant liquor which contains a large share of the gluten. More gluten may be washed from the surface of the starch

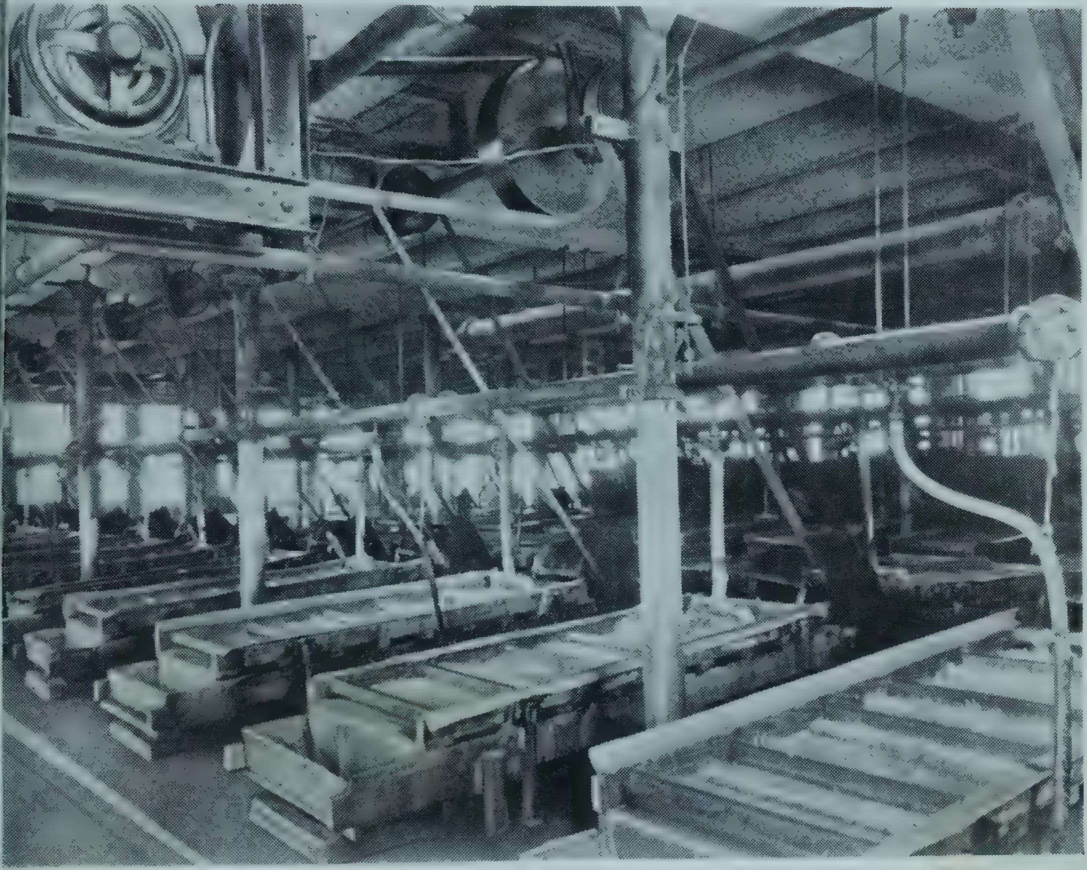


FIG. 38. Shakers.

by the use of a jet of water and gentle agitation. Resuspension of the settled starch is accomplished by adding fresh water and using wooden sweep agitators which are attached by drag chains to a collar around a central shaft in the vat so that the agitator blades may float and remain free of the settled starch. The efficiency obtained in separating starch from protein by this method is poor.

A second method of separation is to introduce the slurry of starch and gluten continuously into the top of a cone shaped vat and to draw off the starch as a concentrated slurry at the bottom. Separation of starch from protein is not as complete as is demanded by present day standards. However, the general principle of a continuous gravity separator is sound and the application deserves further study, taking advantage of progress that has been made in the development of continuous separators or concentrators in other industries.

The principles of flotation which have been developed and applied with considerable success in recent years, particularly in the purification of ores,

FOOD TECHNOLOGICAL RESEARCH INS
LIBRARY

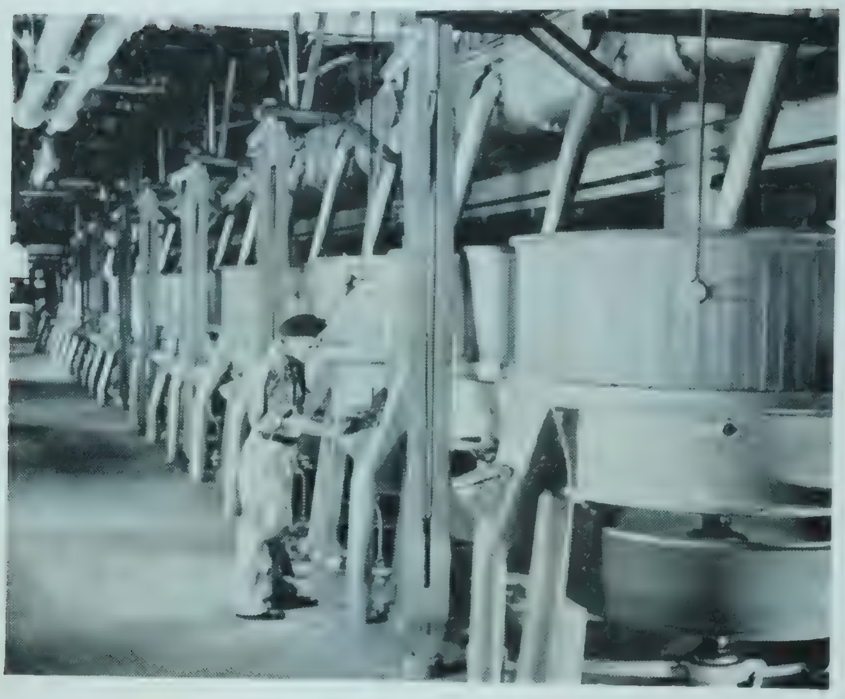


FIG. 39. Buhr mills.

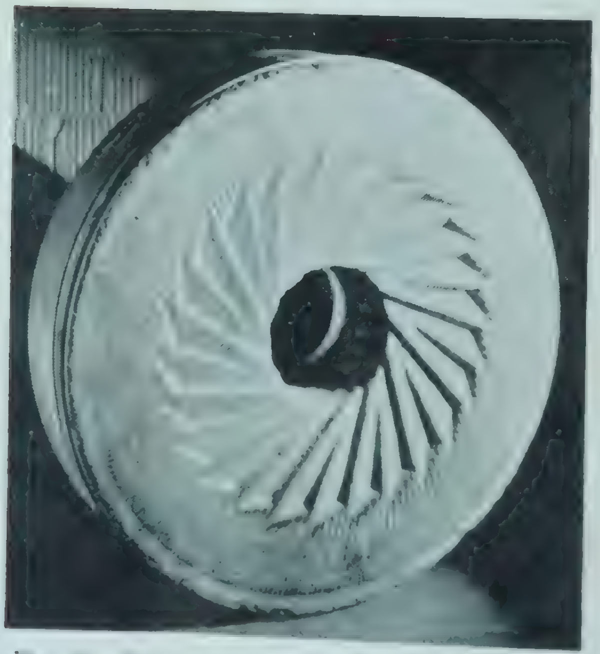


FIG. 40. Stone grinding surface of a buhr mill

has been applied to the separation of starch from gluten. By the use of selected frothing agents and depressents, either the gluten or the starch may be floated. At least one large mill in the United States employed this method commercially in the past. However, knowledge of the principles employed were then in a preliminary state of development and present day, efficient flotation agents were not available. The process was abandoned many years ago and tables were installed. Several patents (34-39) have been issued which illustrate the application of flotation to the separation of starch.

Sedimentation of starch from gluten by allowing the liquor to flow continuously along an inclined trough or table, as it is called, is now almost universally employed to make the separation. The dimensions of the table, the inclination used, the rate of flow employed and the results obtained are, to a large extent, determined by the characteristics of the slurry of starch and gluten employed. An important feature is the density. In the early days rather dilute suspensions were used corresponding to about 3°Bé. This value was raised to 6°Bé. when the industry "bottled up" the wet milling process, that is, re-used all process waters either in the same or another cycle of operations and discarded no liquors to the sewer. However, the use of liquors for tabling having a density even as high as 6°Bé. requires the handling of about 30 gallons of water per bushel of corn milled in the mill and table house. Since, of this 30 gallons, only about 10 gallons may be passed on to the steep house, such a process requires the re-use of about 20 gallons of liquid for each bushel of corn processed.

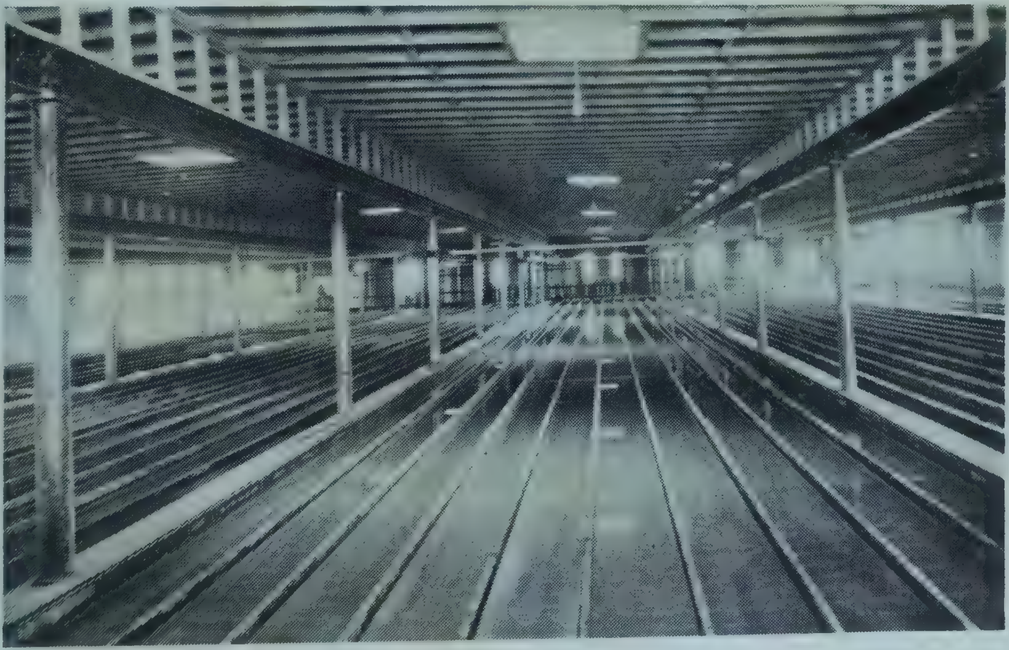


FIG. 41. Starch tables.

Obviously, this re-use of liquors, which contain substantial quantities of water soluble and fermentable corn solids, makes more difficult the production of starch, gluten, and other products of high quality free from the split-products of degradative reactions caused by organisms or enzymes. A discussion of some of the problems involved in the re-use and re-cycling of process waters will be found in patents issued to Widmer (40), McCoy (41, 42), McCoy and Sjostrom (43), Moffett (44), Greenfield (45), Jeffries (46-49), and King and Baker (50).

Considerable study of the use of suspensions of starch and gluten at a higher density has been made in order to eliminate, as far as possible, the re-use of process water in the mill and table house. If densities corresponding to 12 to 13° Bé. are employed, only 10 to 12 gallons of liquor per bushel of corn are needed in the mill house and most of this liquor may be passed on to the steeps (51). All other conditions equal, the lower the density used the more easily is starch sedimented from gluten. In order to maintain a high degree of purity and high yield of starch, or to increase these values as the industry has raised its standards and, at the same time, to increase the density used for separations required a careful study and adjustment of other variables of the process. Kerr (52) has pointed out the influence of several important ones which are: (a) physical state of the gluten, (b) pH of the slurry, (c) ionic strength of the liquid, (d) temperature of the slurry, (e) the rate of flow of the liquors delivered to the table head, and (f) the characteristics of the table, such as ratio of width to length, smoothness of the table bed, and elevation per unit of length.

The desired physical state of the gluten is attained by proper steeping and milling. A satisfactory range of acidity for tabling is between pH 3.8 and 4.2. Separations become more difficult if the pH is displaced outside of this range, e.g., pH 3.0 or pH 5.0. A total concentration of the electrolytes common to mill liquors corresponding to about 50 to 150 grains of ash per gallon of liquid appears to be sufficient. If the value is less than this, the starch, particularly the smallest granules, has a reduced sedimentation rate, so that at certain rates of flow on the tables this part of the starch passes off with the gluten. If the rate of flow is reduced enough to recover the starch, then gluten will sediment on the table to an unpermissible extent. Highly ionized acids or bases are able to increase the sedimentation rate of starch, an effect observed for potato starch by Wiegel (53-54). Therefore, at a very low pH, e.g., 1.5, or at high pH, such as 10 to 11, starch may be tabled because the favorable influence of increased ionic strength contributed by the acid or base counterbalances the adverse effect of a change of pH away from the optimum range. Within operating limits (75-110° F.), the higher the temperature the greater the efficiency of table separation since an increase in temperature reduces the apparent density of the table liquor.

Obviously, if a slurry of starch and gluten is passed over a table at too fast a rate some starch will pass with the gluten fraction, and if the rate is too slow, gluten will sediment with starch. The range of rates of flow at which starch is obtained at the highest purity and yield is very narrow and must be determined for each set of operating conditions. The optimum rate should be maintained by automatically controlled devices for delivering the mill starch to the table head (55-56).

The rate at which the mill starch moves down a table in unit volumes per unit of table length per minute is determined by the delivery at the table head in unit volumes per minute, the pitch or elevation of the bed, and other characteristics of the bed, such as its width. If the width is relatively large compared to the delivery rate, so as to permit a zig-zag course, then the time required for a given volume of liquor to move from one end of the table to the other may be considerably more than anticipated. For an elevation of 1/16 inch per foot, table width should not exceed 1 foot for each gallon per minute of 12°Bé. mill starch delivered to the table head. Unfortunately, the actual pitch of the bed over which the table liquors run does not remain constant during the 3- to 4-hour period normally employed for sedimenting the starch. The reason is that most of the starch in the liquors sediments on the first third of the table's length. Therefore, starch is allowed to accumulate on the table until the speed with which a unit volume of liquor flows down the table becomes such that the recovery of starch falls below an allowable level.

A table length of 120 feet will be found sufficient for one which is 2-feet wide and onto which starch liquor of 12°Bé. is delivered at a rate of 2 gallons per minute. After the desired amount of starch has been deposited on the table, the flow of mill liquor is stopped, a period for draining is given and the last of the gluten is flushed from the surface of the starch with a small water hose. Frequently a squeegee is employed to facilitate this operation and for this reason the mixture of gluten and the rather appreciable quantity of starch which is removed at this stage is often referred to as squeegee starch.

Finally, the starch is removed by the flow of water from rather large jets of water under a high pressure (57). The water used is from the filters to which the flushed starch is now passed for dewatering.

Several types of filters are used for dewatering and washing the starch. The American Filter is most commonly employed. This consists, essentially, of a hollow disk covered with canvas. The disk is divided into sections so that suction may be applied to sections as they revolve and pass through the filter bed and while wash water is applied to the filter cake by means of sprays. Pressure is applied to sections when the starch is being removed by means of a scraper. The Oliver filter and other types of drum filters, such as the Feinc filter, are also used. The Sweetland filter press is employed occasionally. Centrifuges are also employed for washing and dewatering the starch.

Filtration is facilitated by heating the slurry to about 130°F. and the spray water used to wash the starch on the filter may be 135-140°F. In general, starch in a slightly acidic state, e.g., pH 4.0, filters more rapidly than when the pH is in a higher range. The correct range is normally maintained by the addition of a small amount of SO_2 at the first washing filter. The SO_2, which also serves the purpose of protecting the starch from bacterial contamination during drying, is driven off when the starch is heated in the driers. Filters reduce the water content of starch to about 45%; high speed centrifuges reduce the moisture content to 33-35%.

The gluten fraction from the tables is passed to sedimentation tanks, called settlers, or to other suitable concentrating devices. The overflow water from the gluten is used for steeping.

Other systems used industrially for the separation of starch involve the use of centrifuges. Centrifugal separation of starch was proposed in a patent issued to DeCastro and Muller (58). Kerr (59) and Schrader (60) developed the method and Peltzer (61-66) and Boie (67) devised equipment which appears to be particularly adapted to the centrifugal separation of corn starch from corn gluten. In the system described by Peltzer, a slurry having a comparatively high density, e.g., 12°Bé., is delivered continuously to the rotor from whence a substantial portion of the underflow of starch, plus additional liquor, is continuously returned to the centrifugal chamber in order to assist in carrying out the centrifugally separated starch particles. Several centrifuges of this type, frequently referred to as Merco centrifuges, may be used in series. That is, the underflow of starch may be further refined in a second centrifuge and the overflow of gluten may be treated in a third centrifuge in order to recover additional quantities of starch. The latter is composed of relatively small starch granules and contains from about 1 to 10% protein. It is similar in composition to the squeegee starch mentioned above. Other types of centrifuges have been employed, for example, the continuous DeLaval centrifuge and the intermittently fed, basket type. A novel milling system involving the use of centrifuges has recently been proposed by Peltzer (68). In this, fiber and fine grits are not removed from the grist obtained from the buhr mills, but rather, the mill liquors pass to a series of centrifuges. A large share of the fiber passes with the overflow which contains, primarily, the gluten fraction; the remainder of the fine grits is removed from the starch by passage of the centrifuged starch over silk sieves before filtration. One of the principal advantages claimed for this system is a material reduction in the content of water soluble material in the process liquors in the mill houses. A filter is interposed between the mills and the centrifuges and the primary filtrate passes directly to the steeps. The wash water used on this filter is used in the milling operations. The filter cake is repulped to form a slurry of suitable density for use in the centrifuges, using, in part, the overflow water from the first centrifuge. The starch fraction receives a wash in the centrifuge by feeding in water obtained from washing the starch on the final dewatering filters. The primary filtrate obtained from the final dewatering filters is used both to wash the grits on the silk sieves which follow the centrifuges and to repulp the mill starch for preparing the feed to the centrifuges. A gluten slurry is obtained of sufficient density so that it may be passed directly to filter presses without the use of gluten settlers or other equipment for concentration.

The patents issued to Kelling describe the use of centrifugal separation in modern mill practice (69-75). Centrifuges may be used in combination with tables. Staley (76) has patented a process wherein table separation is followed by centrifugation. Many mills now employ continuous centrifuges in order to recover additional starch from the gluten as it leaves the tables and in order to obtain gluten containing a relatively high protein content. By these methods gluten containing 60 to 70% protein and starch containing as low as 0.2% protein are commonly obtained in practice.

The secondary starch, containing the smallest starch granules, may be refined by several procedures investigated by the writer. The starch mixture may be made into a dilute slurry with water and the gluten removed by passage through continuous gravity separators; the protein sediments and the starch, containing about 1% or less of protein, passes out with the effluent liquors.

The replacement of tables by centrifuges in the wet milling industry has been the subject of a recent review and for further study by Taylor (77) and others (78).

Water enters the wet milling system, principally, as moisture in the corn used and as the wash water on the last filter for the starch as it leaves the system. In mills where the starch is converted to sugars, the water used is either softened water or the condensate from various evaporators and boilers. If water is used which contains lime, then the products may be contaminated with gypsum wherever $[Ca^{++}]$ $[SO_4^{=}]$ exceeds the solubility product constant for this salt. The sulfate ion forms unavoidably by the oxidation of the SO_2 which is commonly added at various places in the system, e.g., at the first starch washing filters, at the table heads, at the grit reels, and at the steeps. The water travels in a general countercurrent direction through the table and mill houses and emerges finally as steepwater. The steepwater is evaporated in order to recover the valuable nutritional elements which it contains, such as soluble protein, phosphate, phytate, inositol, and growth adjuncts, and in some cases for the production of industrial chemicals, as by-products of the process. A large proportion of the by-products of the wet milling process, such as, gluten, the germ cake from the oil expellers, the grits, and steepwater, is mixed together in dietetically correct proportions and the product is sold as feed for cattle.

The evaporation of steepwater has presented a problem to the industry in the past because of the heat coagulable material which it contains. The problem has been discussed by Kerr (79), who has offered a practical solution to this phase of the problem. Other methods for treating steepwater prior to or during evaporation have been proposed by Jurgensen (80) and by Schopmeyer (81).

4. Dehydration. After washing and dewatering, the starch may be passed on to various processes applied to modify it, or to converters where the starch is hydrolyzed to make sirups or sugars, or the starch may be dried directly as unmodified corn starch. Formerly, kilns (82) were used almost exclusively for the drying operation. In this, the starch cake is broken up and placed in wagons, such as the Bauer wagon (83-84), or on trays which are then loaded onto a carrier, and these are moved through a heated tunnel or kiln over a period of from 10 to 20 hours. In the Bauer wagon, the sections stand in a vertical position in contrast to trays which are placed on carriers in a horizontal position. Rotary driers are used, particularly for the rapid and continuous drying of modified starches. The Huhn drier is used for reducing the moisture content from normal levels of 10 to 12%, such as is obtained in kiln drying, to 5 to 7%. The latter operation is employed, for example, prior to dextrinization of starch.

Modern practice makes use of continuous belt driers, such as, for example, one manufactured by Proctor and Schwartz. In the final filtration, the starch is accumulated on a revolving drum of the Oliver type, so as to form a sheet 0.25- to 0.50-inches thick. The sheet is scored so as to break into ribbons about 1-inch wide (85). These ribbons fall onto breakers which form lengths of about 2 inches. These sections of filter cake, which are relatively uniform in size, are deposited onto an endless belt made of bronze wire, which carries the starch through a tunnel or series of heated chambers during a period of from 30 to 45 minutes. Heated air is blown through the bed of starch which is about 1.5 to 2.5-inches in depth. The temperature employed, the speed of the belt, and the depth of starch on the belt are mutually interdependent variables. These are adjusted so as to obtain the final desired moisture content for the volume of starch which it is desired to pass through the drier per unit time. The air temperature in these driers varies from about 120 up to about 285°F. In the last section, a forced draft of cold air is applied in order to cool the starch. This procedure has certain advantages over older methods of drying, all of which result in obtaining a starch degraded to a minimum extent in the final operation. A reduction in drying time to less than one hour very nearly obviates the necessity for adding SO_2 to the starch wash water in order to protect it from bacteriological contamination during a long drying period. A reduction in the acidity of the starch and a reduction in the time the acidified starch is exposed to higher temperatures, reduces the loss in paste viscosity normally experienced in kiln drying. Local overheating of the starch is much less when the endless belt type of drier is used than when kilns or rotary driers are used. Very frequently starch is found in some sections of the wagons emerging from kilns having as little as 2% moisture content while in other sections the starch will contain 18 to 20% moisture.

Starch from the driers is crushed to a pearl form or it is milled, for example, by the use of rollers as in the Pope Mill, then bolted through silk sieves in order to obtain a powdered form.

The lump varieties of starch, the so-called crystal forms, are made by two general methods. A slurry of starch is dewatered either by gravity or suction in a box type of filter lined with canvas. The cubes are placed in crusting kilns during which treatment the starch partially dries. Thereafter, the crust is removed and discarded; the core of the cube is dried to normal moisture levels and then broken into the desired particle size. An alternate method has been patented by Moffatt (86). Moist starch is pressed so as to form a cake by the use of a heated cylinder provided with a piston for applying pressure. The cake is allowed to age and is then dried to a normal moisture level, after which it is broken into the desired particle size.

BIBLIOGRAPHY

1. Kingsford, T., One Hundred Years of American Commerce, ed. by C. M. Depew, New York, p. 457 (1895).
2. Szego, E., *Bull assoc. chim.,* **63**, 211 (1946).
3. U. S. Tariff Comm. Report 138 (1940).
4. Schollenberger, J. H., and Jaeger, C. M., *U. S. Bur. Agr. Ind. Chem. ACE 121,* 1 (1943)

5. Hitchcock, A. S., Manual of the Grasses of the U. S., Washington, D. C. (1935).
6. Kerr, R. W., Chemistry and Industry of Starch, New York, p. 85 (1944).
7. Bartling, F. W., *American Miller,* p. 26 (December 1939).
8. Earl, F. R., Curtis, J. J., and Hubbard, J. E., *Cereal Chem.,* **23**, 504 (1946).
9. Hansen, D. W., Brimhall, B., and Sprague, G. F., *Cereal Chem.,* **23**, 329 (1946).
10. Bartling, F. W., *American Miller,* p. 24 (February 1940).
11. Early, E. B., and DeTurk, E. E., *J. Am. Soc. Agron.,* **36**, 803 (1944).
12. Baur, F. J., Jr., and Brown, J. B., *J. Am. Chem. Soc.* **67**, 1899 (1945).
13. Kummerow, F. A., *Oil & Soap,* **23**, 167 (1946).
14. Thaler, H., and Broseff, W., *Fette u. Seifen,* **50**, 513 (1943).
15. Kerr, R. W., in Starch and Its Derivatives, ed. by J. A. Radley, 3rd. edition (in press).
16. Cox, M. J., MacMasters, M. M., and Hilbert, G. E., *Cereal Chem.,* **21**, 447 (1944).
17. Milner, M., *Feedstuffs,* **19** (5), 30 (1947).
18. Semeniuk, G., and Gilman, J. C., *Proc. Iowa Acad. Sci.,* **51**, 265 (1944).
19. Nagel, C. M., and Semeniuk, G., *Plant Physiol.,* **22**, 20 (1947).
20. Bartling, F. W., *American Miller,* p. 24 (April 1940).
21. Killinger, J. E., Dissertation, Iowa State College, Ames, Iowa (1938).
22. Feigel, F., *Z. anorg. u. allgem. Chem.,* **203**, 57 (1931).
23. Olcott, H. S., Sapirstein, L. A., and Blish, M. J., *Cereal Chem.,* **20**, 87 (1943).
24. Henry, W. F., and Bishop, J. P., U. S. Patent 2,322,413 (1943).
25. Bergquist, C., U. S. Patent 2,228,717 (1941).
26. Bartman, L., U. S. Patent 1,670,015 (1928).
27. Eckland, E., U. S. Patent 1,000,726 (1911).
28. Stratton, S. T., U. S. Patent 22,460 (1858).
29. Kingsford, T., U. S. Patent 280,044 (1883).
30. Wagner, T. B., U. S. Patent 2,192,212 (1940).
31. Wagner, T. B., U. S. Patent 2,284,239 (1942).
32. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, p. 20 (1944).
33. Jeffries, F. L., U. S. Patents 1,007,784-5 (1911).
34. Berrigan, J. L., U. S. Patent 994,497 (1911).
35. Holden, H. C., U. S. Patent 1,221,990 (1917).
36. Brindle, R. G., British Patent 159,838 (1922).
37. Feltzer, A., U. S. Patent 2,039,605 (1936).
38. Wagner, T. B., U. S. Patent 2,132,251 (1938).
39. Giesecke, F. O., U. S. Patent 2,251,448 (1941).
40. Widmer, J. M., U. S. Patent 1,585,452 (1926).
41. McCoy, R., U. S. Patent 1,651,611 (1927).
42. McCoy, R., U. S. Patent 1,832,229 (1931).
43. McCoy, R., and Sjostrom, O., U. S. Patent 1,828,334 (1931).
44. Moffett, G. M., U. S. Patent 1,655,395 (1928).
45. Greenfield, R. E., U. S. Patent 2,023,999 (1935).
46. Jeffries, F. L., U. S. Patent 2,050,330 (1936).
47. Jeffries, F. L., U. S. Patent 2,065,313 (1936).
48. Jeffries, F. L., U. S. Patent 2,088,706 (1937).
49. Jeffries, F. L., U. S. Patent 2,122,647 (1938).
50. King, P., and Baker, R. D., U. S. Patent 2,237,561 (1941).
51. Pulfrey, A., Kerr, R. W., and Reintjes, H. R., *Ind. Eng. Chem.,* **32**, 1483 (1940).

52. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, p. 34 (1944).
53. Wiegel, E., and Schüler, H., *Kolloid Z.*, **71**, 65 (1935).
54. Wiegel, E., *Z. Spiritusind.*, **58**, 205 (1935).
55. Curry, H. W., U. S. Patent 1,977,792 (1934).
56. Shurbock, S., U. S. Patent 2,066,142 (1936).
57. Lenders, A. W. H., U. S. Patent 1,456,243 (1923).
58. DeCastro, J., and Muller, T. H., U. S. Patent 273,128 (1883).
59. Kerr, G. A., U. S. Patent 471,614 (1892).
60. Schrader, R., U. S. Patent 757,778 (1904).
61. Peltzer, A., and Peltzer, A., Jr., U. S. Patent 1,923,454 (1933).
62. Peltzer, A., and Peltzer, A., Jr., U. S. Patent 1,923,455 (1933).
63. Peltzer, A., and Peltzer, A., Jr., U. S. Patent 1,933,119 (1933).
64. Peltzer, A., and Peltzer, A., Jr., U. S. Patent 1,945,786 (1934).
65. Peltzer, A., and Peltzer, A., Jr., U. S. Patent 2,013,668 (1935).
66. Peltzer, A., U. S. Patent 2,097,531 (1937).
67. Boie, H., U. S. Patent 2,124,284 (1938).
68. Peltzer, A., U. S. Patent 2,316,807 (1943).
69. Kelling, A. H., U. S. Patent 2,100,744 (1937).
70. Kelling, A. H., U. S. Patent 2,103,217 (1938).
71. Kelling, A. H., U. S. Patent 2,114,497 (1938).
72. Kelling, A. H., U. S. Patent 2,115,170 (1938).
73. Kelling, A. H., U. S. Patent 2,115,171 (1938).
74. Kelling, A. H., U. S. Patent 2,124,779 (1938).
75. Kelling, A. H., U. S. Patent 2,133,543 (1938).
76. Staley, A. E., U. S. Patent 2,116,610 (1938).
77. Taylor, R. L., *Chem. Inds.*, **62**, 54 (1948).
78. Anon., *Chem. Inds.*, **61**, 378 (1947).
79. Kerr, R. W., and Berlin, H., U. S. Patent 1,918,812 (1934).
80. Jurgensen, R. P., U. S. Patent 2,298,623 (1942).
81. Schopmeyer, H. H., U. S. Patent 2,302,393 (1942).
82. Merrill, J. J., U. S. Patent 1,183,097 (1916).
83. Bauer, L. P., U. S. Patent 1,035,302 (1912).
84. Bauer, L. P., U. S. Patent 1,061,720 (1913).
85. Tiers, L. P., U. S. Patent 2,327,943 (1943).
86. Moffatt, A. U., U. S. Patent 541,125 (1895); U. S. Patent 541,941 (1895).

CHAPTER III

MANUFACTURE OF MODIFIED CORN STARCHES

After the native starch has been prepared, as outlined in the preceding chapters, it is frequently modified or altered in some characteristic to make the starch more suitable for some particular use. These modifications vary in degree from those in which there is practically no chemical change in the molecules of the starch granule to those in which a significant change can be detected. Indeed, so varied are the treatments in modern manufacture that it is extremely difficult to classify them and to present a description of the processes in an organized manner. Frequently, moreover, the type of treatment used represents the initial phases of chemical reactions which will be discussed elsewhere. But inasmuch as the alterations to be described are limited mainly to change in physical properties, that is, the resulting product is still essentially starch, they will be grouped under the heading of modified starches.

The treatments to be described apply particularly to corn starch. Many, however, are not limited to this starch, but conditions of operation would quite likely need to be adjusted when the treatment is applied to other types of starches to produce similar effects.

1. **Starches Modified in Color.** Native corn starch from yellow corn exhibits a light yellowish tinge in reflected light, the color arising principally from carotene, xanthophyll, and related pigments. Most of this color resides in the small amount of corn gluten associated with the starch, as finally separated. It is not considered commercially practical to reduce this residual gluten below a value of 0.25 to 0.30% (estimated as protein, by multiplying the Kjeldahl nitrogen value by 6.25). Quite naturally, therefore, the more efficient the separation, the lighter the color of the final starch product. Furthermore, the color of the starch may be lightened materially by procedures designed to reduce the protein content. This may consist of simply retabling the starch by suspending it in fresh water at about 12° Bé. and passing it at a relatively fast rate over a second set of starch tables. A more effective procedure, however, is to stir starch from the first tables with water and adjust the pH to about 9.5 with sodium hydroxide. After being stirred for several hours at a treating temperature of about 110° F., it is then retabled, neutralized, and thoroughly washed.

Daly *et al.* (1) have claimed that if the treatment with sodium hydroxide is made in a range of pH 10-12 and extended for from 5 to 25 hours, a top superficial layer of gluten may be washed from the starch after settling. The oil content of the starch is reduced from 0.5 to 0.3% and the protein from 0.30 to 0.18%, according to these workers. Furthermore, they also claim that after the treated starch is washed and adjusted to pH of 5.6 the clarity of the pasted starch is very much improved.

Schoch (2) has patented a process for the purification of starch which involves refluxing in a water-miscible organic solvent, for example, alcohol, in successive operations. Lipids in the starch are removed and with these the colored bodies from corn and possibly other cereal starches as well. This method possesses certain distinct advantages for purifying starch for some uses, to a degree of purity which cannot be readily attained by other means. However, for producing a white starch for general industrial use, it is a relatively costly procedure, compared, for example, to chemical bleaching.

If the purpose of the treatment is primarily to whiten the starch, then as reported earlier (3) the starch may be washed in alcohol-water mixtures of the order of 60 to 65% alcohol at, or slightly above, room temperatures and the remaining alcohol with the starch may be very efficiently displaced with water and recovered from the wash by distillation. However, recent price levels of industrial alcohols have made this process none too attractive.

A very white product can be made from many varieties of starch by the use of a chemical bleach. Oxidizing agents are quite effective for this purpose. Nevertheless, although a bleach is used, it is always desirable first to reduce the gluten content of the starch to low levels. Otherwise, instead of a brilliant white, grayish tints are likely to result after the bleaching operation.

Corn starch is not bleached as readily in the dry state as might be anticipated. For example, it is the common practice in dry milling to bleach wheat flour with such reagents as nitrosyl chloride, nitrosyl sulfuric acid (4), chlorine dioxide, benzoyl superoxide, nitrogen dioxide (5), diaromatic peroxides such as di-*o*-toluyl peroxide (6), and similar reagents. It is impractical to use these bleaches on dry corn starch, because such large quantities are required to make a substantial improvement in color that the unpleasant odors and taste of the reagents are left with the starch, and in addition the starch may undergo a measurable change in paste properties. Of many dry bleaches examined by the writer only ozone is worthy of mention. This reagent must be used under carefully controlled conditions to secure results approaching those obtainable by wet bleaching methods.

Dry bleaching of starch possesses certain inherent disadvantages. It is the common practice therefore to bleach corn starch in the wet state, *i.e.*, before the final drying operation, so that the chemicals in excess of those actually required to produce the desired effect may be neutralized, and the products of the reactions may be washed away to leave a pure starch. Two of the most common wet oxidants used are chlorine or hypochlorite and permanganate. The former has the advantage in cost but requires, for best results, special equipment for handling, which is either non-corrosive or which does not produce products of corrosion that will be taken up by the starch. Bleaching with hypochlorite has the added disadvantage of producing a noticeable change in the paste characteristics of the starch. Also, the action of the reagent is likely to impart a noticeable foreign odor and taste to the starch unless the reaction is very carefully controlled. Furthermore, to produce a starch of extreme whiteness, it is necessary to purify the starch to a very high level before applying the reagent.

On the other hand, the action of potassium permanganate is so highly selective in its oxidizing action on the colored impurities of corn starch that very small amounts of reagent are required. The type of starch-treating tanks, common in most mills, may be used, and no change in starch paste characteristics can be detected after the reaction. A brief description of the process from the patent of Sjostrom (7) follows.

A water mixture of corn starch is made up at about 16° Bé. For each 1000 lbs. of starch treated, 0.8 lb. of potassium permanganate, dissolved in about 4 gals. of water, is added. After being stirred for about 30 min., the mixture turns to a deep tan color, indicating the end of the primary reaction.

About 1.1 lbs. of sulfur dioxide are then added, made by treating 1.7 lbs. of sodium bisulfite dissolved in 3 gals. of water with 0.4 lb. of sulfuric acid at 66° Bé. The deep colored manganese peroxide, a product of the primary reaction of the permanganate on the carotene and xanthophyll, is reduced by the sulfurous acid to manganous sulfate, leaving the mixture finally a pure white color.

The small amount of sulfurous acid in excess is neutralized by adding sodium carbonate until the pH of the liquor is raised to 5.5 to 6.0. The starch is then filtered and the soluble reaction products removed by washing.

The starch is dehydrated, ground, and bolted.

Other methods for bleaching starch have appeared in recently issued patents. Eble and Mason (8) have patented the bleaching of starch in aqueous suspension at a pH of 4 to 7 with a hypochlorite, such as NaOCl, and the salt of a chlorous acid at a temperature of 85 to 125° F. Hampel (9) has been granted patents for the bleaching of carbohydrates with chlorites, such as the sodium or calcium salts, in conjunction with aldehydes in aqueous media of not less than pH 7, and also with chlorites in combination with persulfates (10). Vincent (11) has claimed that starch may be effectively bleached in the dry state by intimately mixing with a chlorite which is activated by an acid.

Chemical bleaching, under certain conditions at least, has the added advantage of sterilizing the starch. This is very desirable for some uses, for example, in medicaments and in foods which are to be preserved, particularly canned foods. Vincent has claimed that his process for bleaching starch with chlorite also sterilizes the starch. The use of peroxides, mixed into the starch during the drying operation, has also been claimed for this purpose (12). Kerr (13) prefers the use of chlorine in acid media so as to obtain a minimal oxidation of the starch and to provide a maximal amount of free chlorine for sterilization, particularly for highly resistant thermophilic spores. Bulfer (14) has reported that sterilization may be accomplished by heating dry starch for 3 hours at 122° C. The starch should first be carefully reduced to a moisture content of 5% or less, otherwse the starch may be damaged during the high temperature treatment, and sterilization in respect to thermophiles may be incomplete.

2. **Odorless Starches.** Whereas whiteness is desired in some industrial applications when starch is used as a size, *e.g.* in white paper manufacture, freedom from odors common to starch is desired in certain instances when starch is an ingredient of a food product. For example, powdered or confectioners' cane sugar shows a marked tendency to cake or lump in storage, particularly in damp climates. Starch, added in very small percentages, is very effective in maintaining the powdery condition of the sugar, especially when the starch is predried to low moisture content. But when starch is overdried, for example to about 5% moisture, certain reactions are induced in the constituents of the corn starch granule, so that in time the starch develops an objectionable odor which might be described as similar to rancid oil.

Obviously, these odors might be prevented by extracting the fatty constituents of starch granules with a fat solvent such as hot alcohol, which likewise would also produce a starch of brilliant whiteness. It has been found by the writer that such odors may be prevented from forming and at the same time a starch of superior whiteness be produced by the judicious use of an oxidizing agent followed by a prolonged drying action (15).

A description of the method of treatment follows.

Carefully washed starch is made up to 16° Bé. with fresh water and, for each 1000 gals., 2 lbs. of potassium permanganate dissolved in about 4 gals. of water are added. The mixture is stirred for about 30 min. at about 100° F. Sulfurous acid is then added in excess to solubilize the manganese peroxide by changing it to manganous sulfate. The products of the reaction and the excess sulfurous acid are then removed by washing. The starch is filtered and carefully dried to about 5% moisture content, ground, and bolted through silk. Normally such a starch has an acidity corresponding to a pH between 4.0 and 4.4. It is now heated with constant agitation, such as in the common type of dextrin cooker, at 180° F. for about 20 hours.

The starch is then cooled, rehumidified to 5% moisture, and packed in suitable containers. It is claimed that such a starch will not develop the characteristic rancid odor given by cereal starches of low moisture content stored for prolonged periods, because the unstable fatty constituents are oxidized in part by the permanganate and the reaction is completed by atmospheric oxygen at higher temperatures. A part of these degraded constituents is removed in the washing, a part is volatilized in the heating, and those that remain are apparently stable against further deterioration. Other oxidizing agents may be used to initiate the oxidation of the fatty constituents; *e.g.*, chlorine, ozone, and so forth.

3. **Mobile (Dry) Starch.** Certain users of dry starch prefer a powdered product, referred to in the trade as a mobile starch. The reasons for one starch being mobile and another not are not clearly understood, and probably a satisfactory definition to cover all phases of mobility could not be given. Evidence of mobility in a starch is shown by one or more of the following characteristics: (*a*) The starch shows an abnormal tendency to create dust when agitated. (*b*) It will adhere and spread more evenly than normal starches over certain surfaces on which it is dusted. (*c*) If loosely packed into a container it will occupy a greater volume per unit of weight. (*d*) If it is placed on a very fine mesh screen, a larger percentage of starch will pass through the screen for a given amount of agitation of the screen. Some of these characteristics have been used as a basis for a quantitative test to measure relative mobility. These tests are described in another section.

The problem has been critically studied by the writer who has found that among others the following may be listed as the principal variables contributing to lack of mobility: (*a*) high moisture content, (*b*) high percentage of ether extractives, (*c*) high percentage of water extractives which develop tackiness when exposed to a humid atmosphere, (*d*) electrostatic charge, (*e*) shape and size of the starch grains or aggregates, (*f*) prolonged heating in the dry state.

Several attempts have been made to induce mobility in a starch, some of which have appeared in the literature. The earliest method used probably was a thorough washing of the starch by soaking in dilute sodium hydroxide followed by partial neutralization, at least, and then using a copious supply of warm water to complete the washing process. Some improvement will be noted, particularly if the starch is left on the alkaline side, owing possibly to a more complete removal of extraneous fats, proteins, and water-soluble portion of the corn than in a simple washing with water.

Other types of treatment involve a complete removal of some of the interfering substances by washing with a non-aqueous solvent such as hot alcohol or alcohol-water mixtures.

Still another is to pass a purified and highly dried starch over a grounded, metal screen. A more recent variation of this method is to change the sign of the electrostatic charge on the granule (16).

Finally, air separation has been practiced to secure granules of more uniform size and shape.

In most of these methods, however, there is a failure to take into account the fact that of all the variables listed the moisture relationship, in and around the starch granules, is the most important variable. This should not be interpreted to mean that by the addition of an oil to starch, for example, the starch could not be made as dustless or as easily molded into shape by compression as by the addition of moisture. Starch manufacturers produc the opposite of a mobile starch by just such a procedure for certain uses particularly for use as a molding starch. Indentations are made in a laye of the starch and confections such as gum drops are poured from the can cooking kettle to cool and assume uniform shapes. It does mean, howeve that in consideration of a corn starch of the degree of purity now produced by the larger and more progressive mills, moisture relationships are by far the most important variable. This study is admittedly intimately connected with a study of the art of starch drying, as will be evident from considerations given below.

Wet starch, as obtained from the filters in the last washing stage of starch production, may average around 45% moisture. This large percentage is made up of water held in a variety of ways. It is held by capillary action in the empty spaces of the filter cake, it is held as simple surface moisture on the individual granules, possibly some is in a "free" state inside the granule, and lastly, some is held in a combined state with the molecules of the starch. It is suggested that this latter moisture is held by hydrogen bonding to the otherwise free hydroxyls on the carbohydrate chain, and as such will be referred to as bound water.

Wet filter cake is very immobile. Some 5 or 10% of the moisture, capillary water, can be removed by centrifugation at high speed in a closed chamber. The product assumes a fluffy condition but is still very logy. The next 20 or so of moisture can be removed by careful drying, leaving a starch whose surface moisture is reduced to a minimum and, as first made, the starch is quite mobile but very soon becomes less mobile. If the carefully controlled drying operation is continued, as over H_2SO_4 in a vacuum at 45°C., th starch rapidly falls off in moisture content to 7 or 8%, at which level it becomes surprisingly mobile and, if the drying is extended, the moisture gradually falls off to very low levels, 4 or 5% and a starch of high mobility results. At this stage only a portion of the moisture normally present in air-dried starch remains, and this quite possibly is bound water. If the drying is extended to completion by the use of higher temperatures and longer times, irreversible changes are noted in the properties of the starch. For one thing, the absorption of moisture is not nearly as speedily reversed (Samec, 17). For another, it is noted that the starch soon develops a lack of mobility, characteristic of some of the torrefaction dextrins.

The above facts are interpreted to mean that only capillary moisture and surface moisture of the filter cake have a bearing on the mobility of a starch. Internal free moisture is indirectly related in that there is a speedily attained equilibrium between the latter and the surface moisture. Bound water, however is of no significance as long as it stays bound. The establishment of equilibrium between the most tightly held, bound water and forms of "free" moisture is slow. It might be supposed that the last percentages of bound moisture are held by 6-carbon hydroxyls on the carbohydrate chain, since primary alcohols have greater associative forces than secondary or tertiary alcohols. It might also be calculated by simple arithmetic that possibly as high as 9 to 10% of moisture could be bound in this position, provided each 6-carbon hydroxyl binds 1 molecule of water. More likely, however, the value is probably half of this, owing to the organization of molecules within the granule being such that 1 molecule of water is probably shared by a 6-carbon hydroxyl and some other hydroxyl through another hydrogen bond.

It would seem, then, that all that is required in practice is to dry a pure starch to 5% moisture and mobility would automatically result. Two difficulties are encountered. When the common method of drying the starch in the kiln to about 10%, followed by grinding, bolting, and redrying to 5% in a Huhn type drier is used, the resulting starch is not as mobile as was anticipated from laboratory experiments. Furthermore, soon after manufacture the starch becomes less mobile, which is explained in part by the fact that at normal humidities it tends to increase in moisture content, presumably, at first, on the surface. A more critical examination of the drying process given discloses that at the 10% moisture stage a part of the starch in the carriers in the kiln is severely overdried, having possibly as low as 2% of moisture, whereas another portion may have as high as 18 to 20%. Therefore both of these fractions, that high and that low in moisture, are immobile (although for different reasons). Furthermore, in the redrying process which follows, that portion of the starch which is already too low in moisture content for good mobility is dried to an extent such that it is, in effect, irreversibly modified; a state of equilibrium in the moisture content of this starch at any given humidity can be reestablished only after a long exposure to high humidity or by direct contact with water. In the lot of starch reduced to an average moisture content of 5% it also follows that there must be some granules with moisture contents higher than 5% and these therefore contain a certain percentage of moisture which is in a flexible equilibrium with surface moisture.

Better mobility at a level of 5% moisture content can be secured by more uniform drying. Starch dried in a rotary drier is noticeably more mobile than that dried in a kiln and a flash-dried starch begins to approach that which can be attained with controlled drying in the laboratory. None of these methods, however, is perfect in eliminating local overheating. In addition, drying to a moisture content of 5% in such equipment involves the constant hazard of fire and explosion during the handling of starch low in moisture content. Moreover, the product is likely to regain sufficient moisture, before it reaches the customer, to lessen its mobility materially.

If the above facts are granted and the above hypotheses assumed to be substantially correct, one solution to the problem would be to increase the capacity of the components of the starch granule to hold more bound water: that is, to activate the starch, so to speak, so that the associative forces for water molecules are increased.

It was found by the writer that treatment of starch with chlorine gas, in controlled amounts, produces the desired result. The method is described in a patent issued to Kerr (18).

Table IX illustrates the results obtained. Mobility is measured and expressed as the time in minutes for a given weight of starch to pass the 48 mesh copper wire screen on a shaker sieve attached to an eccentric making 120 r.p.m.

A mobility of 6 minutes and under is considered satisfactory for most uses. Two commercial starches (Samples A and B) were prepared by two

TABLE IX

Mobility of Starches

Type of corn starch	H_2O	pH	Mobility
	%		min.
Chlorine-treated, 8 hrs. at 125° F.	14.4	5.0	2.5
Chlorine-treated, 3 hrs. at 95° F.	8.9	6.7	4.5
Chlorine-treated, 3 hrs. at 95° F.	12.9	6.1	5.75
Chlorine-treated, 3 hrs. at 95° F.	11.3	5.4	6.0
Chlorine-treated, 3 hrs. at 95° F.	12.3	4.9	7.25
Untreated, flash-dried, air separated	8.5	5.5	9.0
Untreated, air-separated	10.0	5.5	14.0
Commercial, 5% moisture	5.0		Over 30.0
Commercial, powdered (Sample A)	10.0		Over 30.0
Commercial, powdered (Sample B)	10.0		Over 30.0

different manufacturers. The chlorine-treated starches were dried in a rotary drier and air-separated. The most highly chlorinated starch felt dry to touch, even at 14% moisture, and the 100 lbs. of starch used in the test fell rapidly through the shakers, filling the surrounding room with dust.

4. **Starches of High Viscosity.** The chief industrial use of starch depends on the fact that, when starch is mixed with water and heat is applied or certain chemicals added, the starch swells to form a paste. An important feature of this paste is its viscosity or, the inverse function, fluidity. Starches of various viscosities are required for various industrial uses. For most uses starch of normal or reduced viscosity is required. Occasionally, however, uses develop in which it is more advantageous to employ starches of very high viscosity, the higher the better. A few examples of these follow. When starch is used as a thickening agent in cooking, as for instance in thickening soups or canned vegetables, other things being equal, the higher the viscosity of the starch the less starch will be required. Another example is its use when an inexpensive adhesive is essential. Paper bags are sold at a price range in which an inexpensive adhesive, for use in forming the seams, is not only desirable but necessary. Obviously, other things being equal, the higher the viscosity of a starch paste at a given concentration, the more it can be diluted with water to a fixed level for use on the bag-forming machines.

Some starches, when cooked into a paste, exhibit higher viscosities than others at high temperatures, that is, around the temperature used in cooking the paste, but either they show no increased viscosity at lower temperatures or else possibly a subnormal viscosity. It does not necessarily follow that, because a starch exhibits an increase in viscosity at one temperature, it will necessarily exhibit the same proportionate increase in viscosity at all other temperatures. Hence it becomes important to describe starch viscosities in terms of temperature and to select the starch for an intended use which exhibits the desired viscosity at the temperature at which it will be used. In practice, therefore, it is customary to speak of hot paste viscosity and cold paste viscosity. The former is often measured by the Scott test at temperatures in the neighborhood of 95°C. A common method of measuring the viscosity of dilute, cold pastes is by means of the Stormer viscosimeter at 25°C. after a stated cooling and aging period. Both of these methods are described in the section on methods which follows.

Several methods exist for increasing the viscosity of corn starch above normal. Some of these, however, possess inherent disadvantages. Perhaps the oldest method is simply to add an alkali or alkaline substance to the starch in sufficient concentration for it to gelatinize with no applications of heat. Very viscous masses result, much heavier in body than obtained by heat gelatinization and cooling to room temperature. Such a paste exhibits a high, cold paste viscosity but rapidly deteriorates into a thin fluid if it is heated at boiling temperatures for any length of time. Obviously the high alkalinity of such a paste is another decided disadvantage for most uses. The alkalinity required to gelatinize starch into a thick viscous mass may be reduced by either partially gelatinizing the starch before addition of the alkaline reagent, or it may be heated in the presence of even less alkali with the production of relatively high viscosities. But in any event such pastes are quite unstable and lose viscosity at a rapid rate at higher temperatures. They thin out with mechanical agitation required to pump the paste from cooking kettles to other equipment and with the movement of mechanisms used to apply the paste.

It will be appreciated, therefore, that the addition of alkalies to starch does not, according to the Scott test, increase the viscosity of a starch above normal, since the viscosity is determined after a definite cooking and holding period at high temperatures.

One of the more acceptable methods of treating corn starch to increase the hot paste viscosity is with chlorine gas or by the addition of small amounts of hypochlorite. Bryant (19) claims increases in viscosity of the starch result from a limited treatment of corn starch with hypochlorite. Kerr (18) discloses that the hot paste viscosity of a starch may be very materially increased by treating an aqueous suspension of raw corn starch with chlorine gas. The hot paste viscosity of the starch continues to increase with the addition of chlorine up to the limit of saturation of chlorine in the starch slurry. When an absorption tower is used, it is found possible to dissolve 2 lbs. of chlorine in 100 gals. of starch slurry containing 300 to 400 lbs. of starch. After a short holding period, without heat, the excess chlorine is then removed by use of an antichlor, and the starch neutralized and washed free of salts. A starch so treated is claimed to increase in hot paste viscosity, expressed in terms of the Scott test, from a normal of 90 to 100 up to as high as 500.

The cold paste viscosity of the starch described is lower, however, than normal. Less extensive treatment with chlorine, while it falls short of producing the peak hot paste viscosity obtainable by this method, does, none the less, develop an increase in cold paste viscosity of the starch above normal for untreated starch. The use of a more strenuous treatment with chlorine than in the illustration given, by extending the time the starch is in contact with chlorine, results in a gradual falling off from the peak hot paste viscosity to normal and, finally, below normal. Secondary reactions evidently become predominant. One of these is, possibly, hydrolysis, owing to the acidity of he reacting medium; another is the oxidizing effect of the hypochlorous ion.

An additional advantage to be gained in the type of treatment for starch lescribed above is that the product is not only improved in viscosity for uch uses as in canning and preserving foods, but also the starch is rendered quite sterile, particularly in respect to resistant organisms, the spores of which are ever present in the atmosphere and which are naturally present on most farm products as they are brought in from the fields for canning. The use of a pure, sterile starch, therefore, lessens the canner's responsibilities in respect to inactivating only those organisms which may be present on or in the other food ingredients, such as the vegetables and condiments, which go on to make up the canned food. Kerr (13) has described a method for producing a superior starch of this type in a recently issued patent.

The viscosity of starches may be increased above normal by treatment with other halogens, such as bromine, as reported by Kerr (18).

Another type of treatment, the fundamentals of which are given elsewhere, is pretreatment of starch in manufacture with aldehydes; *e.g.*, formaldehyde, acetaldehyde, glyoxal, etc. A method for treating starch with formaldehyde has been described by Rowland and Bauer (20) who have patented the use of this type of starch in sizing paper during the beater operation. The method is essentially as follows: 1000 lbs. of corn starch are mixed with 1200 lbs. of water in a suitable tank equipped with an agitator. To this mixture are added 10 liters of a 40% formaldehyde solution and 2 liters of HCl (18° Bé.). The mass is held at about 75°F. and agitated for about 24 hours, or until the desired amount of reaction has taken place. At the end of this time the water is separated from the starch by means of a filter or a centrifuge and the starch is washed several times with clean water. The starch is then dried and is ready for use.

The author and his associates have experimented with a similar process and have noted that the Scott viscosity test of corn starch is raised from a normal of 90 to about 150 or higher by such means. In a limited treatment with formaldehyde the cold paste viscosity of the starch also increases to some extent as in chlorine modifications. As the treatment is extended, with an increase in Scott viscosity values above 150, the cold paste viscosity tends to fall off. But in marked contrast to modification by chlorine, formaldehyde-treated starches show an increased resistance to gelatinization by heat, the pastes are more stable to mechanical agitation and pump pressures, and are much more stable in the presence of alkali than untreated starch. Indeed, formaldehyde-treated starches which exhibit Scott viscosities of about 150 when the pH of the paste is 5.0 actually increase in Scott viscosity as the pH is raised by additions of borax or alkali. Between pH 9 and 11, Scott viscosities of the order of 200 have been observed.

The more extended action of formaldehyde on starch will be discussed elsewhere.

The action of aldehydes on the non-cereal starches, such as tapioca, is unique. By means of a limited treatment, starches may be produced which cook up to cloudy pastes resembling the cereal starches and which set to gels. By inclusion of certain agents in the cooking process, *e.g.* sugar and fruit acid, clear transparent gels result, resembling gelatin desserts. Edible products may be made by the use of acetaldehyde as the modifying agent and elimination of the reagent after modification of the starch by adding bisulfite in excess and by a thorough washing out of the addition products, according to Kerr and Schink (21).

Condensation products of formaldehyde and urea, such as dimethylolurea, are able to act on starch granules so as to produce a modified starch which when gelatinized in water has a very high paste viscosity. Gill and McDonald (22) have patented the manufacture of thick pasting starches, made by these means. As an example, when corn starch is heated in the wet state at 125°F. with 0.2% of dimethylolurea at pH 4.5 for 5 hours and the product is then dried, the hot paste viscosity is increased to a value of 320 by Scott test, compared to a value of 70 for the untreated starch. It was also found that 0.5% addition of the reagent raised the Scott test of the starch to 1000. These workers also found that a monomer of formaldehyde and melamine produced a similar effect and that all of these starches produced smooth pastes.

Within recent years there has been a growing interest in modifying the paste properties of starch by means of a very limited amount of chemical derivatization. These are in reality partial derivatives such as esters and ethers. However, the degree of substitution is so low—much less than an average of one derivatized hydroxyl group per glucose residue—that the products behave in many respects like native starches, particularly in that the granules are insoluble and gelatinize when heated in water to form a paste. The pastes of some of these products differ materially from that of the parent starch. Many from corn starch, for example, have greater clarity, better mobility or less plasticity, some exhibit paste viscosities very much greater than the untreated starch, and some, on cooling, do not set to a firm irreversible gel, so characteristic of native corn starch.

One of the earliest products of this general type was an acetate of very low degree of substitution, prepared by Traquair (23) and was sold under the trade name of Feculose. Latterly, however, many products have appeared on the market which are ethers and particularly those in which additional hydrophyllic groups (24) such as carboxyls (25), are present in the alkyl group which is etherified with the starch. Considering that in many cases hese starch products do not show on analysis more than one substituted roup for every twenty or more glucose units in starch, it seems rather emarkable that the colloidal characteristics of the modified starch should ave changed fundamentally. It would appear that a very small number of ubstitutions on a starch chain, particularly the linear amylose molecules, re sufficient to prevent the orientation of these molecules to the extent equired for associative bonds to form as they do in retrogradation, set-back in pastes and the formation of gels.

When these reactions are extended into the range where the degree of substitution approaches an average of 0.5 per glucose residue, or higher, the organization of the granule is weakened to the extent that the product swells, or dissolves in cold water. These products resemble the high viscosity, natural vegetable gums in their properties, and they differ in minor respects depending on the nature and number of the substituted groups. The reactions used in their manufacture and the properties of these products will be discussed in a following chapter (Chapter XIII) on derivatives.

When bifunctional reagents are used to modify the starch such as, for example, dibasic acids or epichlorohydrin, modified starch products of increased paste viscosity are formed in the early stages of the reaction, according to a patent issued to Maxwell (26). A Dutch patent was recently allowed to Lolkema and Kool (27) covering similar reactions. According to Maxwell, the introduction of a very small number of cross-links between carbohydrate molecules is responsible for this increase in paste consistency.

However, as in the case of the formaldehyde reaction, wherein possibly methylene cross-links are established between starch molecules, as the number of these cross-links increases, the starch gelatinizes with greater difficulty, and finally gelatinization is inhibited. The pastes become very noticeably more short in texture, more opaque, and after an extended treatment, microscopic examination shows that the granules do not swell even after heating in boiling water.

That plasticizers such as sulfonated oils, when added to starch pastes in the preparation of sizes and adhesives, cause a marked increase in the hot paste viscosity, as well as a lowering of the tendency of the paste to gel on cooling, has long been known in practice. That the property is characteristic of all dipolar substances, to a greater or smaller extent, has been established in researches conducted by the author and his associates. The higher fatty acids, such as stearic, palmitic, and linoleic, all materially increase the hot paste viscosity of corn starch when added to the starch in small amounts. The use of soaps to increase the viscosity of starches has been studied by Heald (28) and others. However, the action of most adjuncts, or "plasticizers" is to decrease the consistency of starch pastes, particularly their consistency at room temperature.

5. **Starches of Reduced Viscosity.** While there is a definite market for starches of abnormally high viscosity, most of the modified starches sold are reduced in viscosity below that of native starch. The principal reason is that by so doing more dry substance (starch) may be used in the size, adhesive, or other product, and at the same time the pasted starch product will be fluid enough to be workable. All other factors being the same, the adhesive or binding power, the strength of film, and other desirable properties of this nature increase very materially as the amount of starch increases. The viscosity of a starch paste, however, depends on at least three factors, the apparent viscosity due to swollen granules, the viscosity due to dissolved molecules or dispersed colloidal aggregates, and a viscosity that results from an orientation of these molecules or aggregates. Viscosity may, therefore, be lowered by reducing the tendency of the granules to swell before they rupture, by a reduction in the size of the dissolved molecules, and by a modification of the molecules to reduce their tendency to orient. The second and third methods involve the danger that a decided drop in adhesive strength will follow, owing to a degradation of the starch molecules, for strength in a size or adhesive is, among other things, a function of molecular magnitude. It is obvious, for example, that the lower sugars are weaker as sizing and adhesive agents than the higher dextrins.

A mild treatment with acid is perhaps the most common method to modify starch for this purpose and the products are the so-called thin boiling starches of commerce. The method of treating starch with dilute acid at temperatures below the gelatinization point of the starch to produce these thin boiling starches appears to have been introduced by Duryea (*29*).

The method of manufacture is to suspend starch in dilute sulfuric acid (between 0.1 to 0.2 *N*), warm to about 50 to 55°C., and stir at this temperature until the potential paste viscosity of the suspended starch is reduced to the desired level. The suspension is then neutralized with sodium carbonate, filtered, washed, and dried. A series of products is manufactured showing viscosities from levels only slightly below normal for untreated starch to those low enough to indicate that the product is essentially an acid-modified dextrin. The products are graded on an arbitrary fluidity scale, the higher the fluidity assigned, the lower the indicated viscosity. Untreated starch has a fluidity number of 1, water itself being considered as 100 fluidity for reference.

An alternate procedure used in making the highest fluidity starches is to acidify a suspension of the starch to be treated, filter without neutralizing, and place the moist filter cake in kilns. Conversion usually requires several days time. This method eliminates the loss of dry substance in the form of water-soluble products which arise in an extended acid conversion. In the method described first, the soluble matter produced in the tub conversion passes out with the filtrate at the filters.

Although the thin boiling starches of higher fluidity, such as 75 and 90, exhibit a reducing value large enough to indicate a hydrolytic scission of starch molecules as the result of the acid treatment, hydrolytic degradation is difficult to demonstrate in starches modified as far as the intermediate fluidities, *e.g.*, 40 fluidity. But it should be pointed out that Farrow and his coworkers (30) have suggested that the breaking of a very small percentage of the glucosidic bonds in starch is sufficient to cause a material shortening in the average "chain length" of the molecule.

An examination of a thin boiling starch granule during gelatinization discloses, however, that it swells considerably less than untreated starch. Rather, it disintegrates at an early stage into small fragments (31). It would seem likely, therefore, that the reduction in hot paste viscosity of thin boiling starches is the direct result of the lesser tendency to swell. The reason for the latter may be that there is a limited acid degradation of those constituent molecules on which the tensile strength or elastic limit of the granule mostly depends.

Another consideration favors the view given. It has long been known that while the hot paste viscosity of acid-modified starches is materially reduced, their pastes show a much more decided proportionate increase in viscosity on cooling (32). Indeed so pronounced is the tendency of thin boiling corn starches to set up, or gel, that they are unsurpassed for use in such confections as gum drops. It would appear, therefore, that one effect of the acid is to break off large fragments of some of the branched chain components of the starch, which fragments are sufficiently linear and are of the proper length of chain to orient in paste formation and thus increase the tendency of the starch to gel.

Further support for the explanation proposed is derived from a consideration of the chemistry of potato starch. The conclusion is supported mainly by the fact that potato starch is essentially non-gelling in character and by the claim in a German patent (33) that potato starch may be transformed into a starch which cooks and sets to a short gel by warming a suspension of the starch over an extended period of time with carefully controlled concentrations of acid. Potato starch apparently contains no significant amount of unbranched molecules of the proper chain length for orientation into structures which lead to gel formation, but a very limited scission of some molecules increases the proportion of linear chains of the optimum length, so that when the starch forms a paste, gel formation results.

A second and common method of producing modified starches of low viscosity is treatment of the starch with oxidizing agents. Hypochlorite is quite frequently used for this purpose. This method will be taken up, however, in the section on reactions.

The action of most reagents on starch is attended by a lowering of viscosity. A great many reagents have been used and suggested for use. Even the patent literature is too voluminous to cover completely in this review. Therefore, only a few representative examples will be given.

Salts that can act as oxidants might be expected to accomplish their modification of starch by an oxidizing effect. The patents of Harvey (34), Pierson (35), and others on the use of peroxides and other per-salts are examples of this type. Kerr (36), however, points out an unanticipated effect in the use of calcium peroxide. Whereas most oxidants produce a starch whose cold paste is definitely less congealing when compared to an acid-modified starch of comparable hot paste viscosity, calcium peroxide, on the other hand, produces a starch whose cold paste sets to a gel of greater strength than an acid-modified starch. Obviously such a starch would be more suited for use in making products in which gelling is desirable, as for example in the manufacture of gum drops.

Acid salts, *e.g.* $NaHSO_4$, might be expected to modify starch by virtue of the acid activity which develops when the salt is dissolved in water. Salts of weak bases and strong acids, such as ammonium chloride, calcium chloride, and aluminum chloride, which are easily hydrolyzed in water, might be expected to modify the starch for similar reasons.

Frequently, however, there have been reported modifications of the starch that are due to salt effects but are not attributable to the above causes. For example, if very small quantities of an inert electrolyte are present, *e.g.* sodium chloride, certain starches, when they are gelatinized and allowed to stand, lose viscosity at a rate suggestive of a material degradation in the starch molecules. Corn starch is, however, not very susceptible to this effect, but apparently potato starch is (37). So pronounced is this action, that for many years it was argued by Biedermann (38, 42), Haehn (43), Iljin (44) and others that the ash of saliva might be the active amylotic principle. Haehn and Berentzen (45) concluded that a mixture of neutral salt + peptone + amino acids catalyzed the degradation of starch in a manner analogous to the amylases and were, therefore, the active ingredients of amylases.

Such effects, as noted above, in which starch is apparently "liquefied" by ontact with small amounts of electrolytes are hardly explainable on the basis of changes in osmotic pressure, within and outside of the starch granule. They are not satisfactorily explainable by a weakening effect on hydrogen bonding in oriented colloidal systems for in this case salts such as NaCl should reduce the viscosity of corn starch pastes more than that of a similarly prepared potato starch paste, since secondary bonds appear to be a greater factor in the viscosity of the former than the latter paste but neutral salts have very little effect on the viscosity of corn starch. Further, the results reported would not be explainable by the phenomenon discussed by Zwikker (46), that the alkali salts of amylopectin form less permeable membranes than the salts of the alkaline earths, for when sodium chloride is added to potato starch and thinning results (and if we assume a substitution of the calcium in a naturally occurring calcium salt of potato amylopectin by the added sodium), the amylopectin membranes should become less permeable and more resistant and the granule and colloidal units should show less tendency to absorb water and rupture, finally.

Samec (47) attributes the high viscosity of potato starch to the ionizable amylopectin-phosphoric acid complex. However, the sodium salt of the amylopectin-phosphoric acid should ionize as well, possibly to an even greater extent than the corresponding calcium salt.

Bungenberg de Jong (48) explains the action by assuming that electrolytes discharge the negatively charged starch particles, thereby lowering the electroviscous effect or part of the total viscosity of a solution. It would seem that this hypothesis justifies further consideration.

Pierson (49) has claimed that a fluid and stable vegetable glue may be made by treating starch, *e.g.*, cassava, with 0.2% of $Ca(OH)_2$ in the presence of excess alkali (3% NaOH) in a 33% aqueous suspension of the starch at 150°F.

Some salts (and organic compounds) are specific in lowering the gelatinization temperature of starch and have been used for this purpose. Samec (50) has reviewed the early literature on this subject and the effect is further discussed in the chapter on hydrogen bonding in starch. Thiocyanates (51) and chloral hydrate (52), for example, have long been known to lower the gelatinization point of starch very materially. Some starches are affected by these agents more readily than others. Lejeune (53) has noted a great difference in respect to the extent which various starches swell and gelatinize in various concentrations of sodium salicylate in water at room temperature.

On the other hand, some salts exert an insolubilizing effect on starch or act to repress gelatinization. Even the relatively alkaline sodium carbonate materially represses the gelatinization of some starches at higher concentrations of the salt. Caesar (54) has reported that chromic salts of an aliphatic acid, for example chromic acetate, insolubilize a gelatinized starch paste. Iler (55) has claimed that when trivalent chromium is converted into a complex with fatty acids containing at least 10 carbon atoms, the resulting complex, for example, chromium stearate chloride, converts the starch into a hydrophobic material which imparts water resistance to the product in which it is included. Griffin *et al.* (56, 57) have patented the application of potassium pyroantimonate to starch pastes and an acidification of the latter in order to produce water insoluble size and adhesive compositions.

Quite naturally basic reagents exert a swelling or dissolving action on starch, for example, morpholine, ethylenediamine, and the ethanolamines. Pacsu and Mullen (58) have patented the process for producing a colloidal solution of starch in pyridine, or similar nitrogenous base, by gelatinizing the starch in an aqueous solution of the base and distilling off the water as an azeotrope with the base.

Many amides exert a plasticizing action on starch pastes and some are particularly effective. It would appear that the lower fatty acid amides, at least, act by virtue of their solvating action on the starch. Urea, the diamide of carbonic acid, has long been known and used for its plasticing action on starch pastes, particularly in the field of adhesives. Kunze and Evans (59) have advocated the use of dicyandiamide with starch products to reduce viscosity, plasticity, and possible gelling effects in prepared adhesives. Bauer (60) has patented the use of urea together with a solid acid which acts to stabilize the urea. Glarum and coworkers (61, 62) have covered the use of dimethylolurea to stabilize the paste viscosity of various starch products.

A large variety of starch "plasticizers," gelation inhibitors or antisetback agents have been reported and marketed in recent years. Some of these also are very effective in controlling the increase in viscosity, gelling, or retrogradation effects when gelatinized starch products are cooled and aged. In some cases the chemical nature of the adjunct has not been fully reported. Flovis* (63) (Glyco Products Co.), Flexalyn* (64) (Hercules Powder Co.) and Vanesta* (Vanderbilt Co.) are among several which have been marketed in some volume. Flexalyn is reportedly a glycol ester of a rosin acid. Billing (64) has taken out a patent (to Hercules Powder Co.) on the application of a glycol ester of a rosin acid to starch adhesives. Vanesta is presumably a polyoxyethylene stearate. Favor and Johnston (65) have found that the latter tends to preserve the softness of bread crumb and retard other effects which are characteristic of staling.

---

*Trade marks.

Moore (66) has patented the plasticization of gelatinized starch with a polymerized terpene, and Evans (67) has claimed the process for obtaining non-gelling starch sizes and adhesives by the addition of 0.5 to 2.0% of an alkylolamine soap. Kesler and Rankin (68) have patented a starch adhesive composition adapted to be readily spread on a surface in the form of a thin substantially continuous film in which the starch is gelatinized in an alkaline medium and mixed with a compound of an amphoteric metal and a water dispersible long-chain, bipolar, organic material. Neuman (69) has claimed that the gelling of starch may be prevented by the addition of the reaction product formed when an alkali metal acetate, an alkali metal carbonate, and hexamethylenetetramine are precipitated from aqueous solution by the addition of phenol. The use of glucosides of lower alcohols has been patented by Young (70).

6. Cold Water Paste Products. There is a very definite market for starch products which may be stirred up into pastes or sols with cold water. Quite frequently, in the production of very soluble modifications, the process may be attended by a chemical change in the starch; hence a discussion of the preparation of these products is not logically a part of this chapter.

The most common and perhaps the simplest method of producing a starch product which may be mixed into a paste with cold water is to pregelatinize the starch, or partially gelatinize it, with water and heat and then dry it. Corn starch products of this nature are sold under the trade mark Amijel (71). Similar products prepared from tapioca and potato starch are Tapinol, Tufgel, etc. Their principal use is in sizing paper pulp at the beaters. The starch is introduced into the pulp beaters and the action of the beaters redisperses the starch to effect a sizing of the pulp fibers.

There are several variations in procedures for making such products. The simplest method is to pass starch filter cake, having a moisture content of from 40 to 50% following the milling operation, between heated rolls which are balanced in speed and temperature according to the moisture content of the starch and its ability to gelatinize. The latter characteristics will be determined by the variety of starch used, whether corn or potato, for example, by whether additions have been made to the starch to alter its gelatinization, or by whether the starch may have been premodified by a slight chemical treatment.

A thin, gelatinized sheet is produced which is removed from the roll by knives. The product is then adjusted to the proper moisture content, ground, and bolted.

A variation of the process, by which a more thorough gelatinization of the product is secured, is to feed a water suspension of the starch, *e.g.*, about 4 to

5 lbs. of starch per gallon, to the heated rolls. Another variation is thoroughly to gelatinize the starch product with heat and sufficient water and then spray-dry the product or dry by some means suitable for the economical dehydration of viscous masses. Obviously, other things being equal, the extent of gelatinization during the process will depend on the water available at the point of heating. On the other hand, the more water there is to be removed by evaporation and drying, the more costly the process. To secure a better degree of gelatinization a mild alkali may be incorporated with the starch in small amounts; *i.e.*, sodium carbonate, phosphate, or borax. The latter also serves as a wetting agent when the product is redispersed in water. Other wetting agents may be incorporated when a slight alkalinity is not desirable. Alkalinity serves another purpose, however, in that it tends to reduce the retrogradation effects which follow when starch is gelatinized and dried.

Some retrogradation is always shown by products made from unmodified starch in that when they are mixed with water they do not redisperse into pastes comparable with those from freshly cooked raw starch. For some uses a minor amount of retrogradation is not objectionable, indeed, even desirable, as will be evident from a study of uses. When retrogradation is not desirable, the starch may be first modified to reduce retrogradation effects.

In one commercial process the starch is first modified by a slight acid treatment in aqueous suspension. The mass is then fed to heated rolls. Some hydrolysis in the process is evident from an analysis of the product which shows about 5% reducing sugars estimated as dextrose. One such commercial product, sold under the trade mark Amidex, is used as an adhesive.

To improve the characteristics of starch products that swell in cold water, the addition of lecithin has been advocated by Sichel (72), while Möller (73) suggests the addition of lipids.

An improvement claimed in a Belgian patent (74) is to mix starch that swells in cold water with an etherifying or esterifying agent together with the quantity of alkali necessary for etherification or esterification in the absence of any appreciable quantity of water.

Kesler (75) proposes a novel process of stirring a starch suspension while heating and including an agent such as hexalin which presumably limits the swelling of the granules. Heating is continued until the granules loose their characteristic crosses in polarized light. The water content of the slurry is such that at this point the mass is plastic. Heating is discontinued and the mass is stirred until dry enough to complete the dehydration by transferring to normal starch-drying equipment. According to another patent a similar process (76) involves the use of soaps, sulfonated tallows, and oils in amounts varying between 1 and 30%.

Bauer (77) describes a method of treating mixtures of moist starch and urea with steam and then drying and grinding the mass.

More elementary variations in the process for producing a starch that swells in cold water include the following. Stutzke (78) would spray a starch solution into a superheated chamber, whereas Maier (79) would spray starch into superheated steam. Hoppler and Haake (80) claim a starch swelling in cold water may be made by passing starch of normal moisture content between plates at a pressure of 2500 kg. per sq. cm. and a temperature of 150°C. Gill (81) suggests a process for extruding starch at 13 to 17% moisture, under pressure through orifices 0.012 to 0.026 in. in diameter.

Several radically different methods have been proposed. Kantorowicz (82) has described the treatment of starch with alkali in alcoholic suspension. One hundred parts of pulverized starch are mixed with sufficient alcohol to form a milky fluid. Forty parts of a solution of sodium hydroxide (30° Bé.) are then added and the mixture is allowed to stand for an hour. The alkali is neutralized with acetic acid and the starch precipitate filtered, dried, and ground. It is claimed that the product will give a fluid paste upon the addition of cold water. In another patent, Kantorowicz (83) describes the use of acetone or mixtures of ether and alcohol in place of the alcohol. Dritter (84) describes treating starch suspended in a halogenated organic solvent, adding sodium hydroxide, removing the solvent, and then neutralizing. Supf (85) has patented the gelatinizing effect of thiocyanate. He would treat starch in an alcoholic solution of thiocyanate and then wash the starch thoroughly with alcohol. French and Gill (86) described a process for heating starch in glycerol. If carried too far, as for example a prolonged heating at 200°C., then, as shown by the older work of Pictet (87), the product passes beyond a soluble starch stage, and depolymerization reactions produce substances which Pictet called hexosans.

Finally, as an illustration of how extensively early investigators explored the field of making starch products which disperse in cold water, attention is directed to several early patents of Anderson (88-90). The essentials of the process are to treat starch (air-dried) in a closed vessel for 10 to 45 min. at temperatures varying between 125 and 300°C. The pressure is suddenly released, which causes the granules to swell and burst. This action is probably due to the release of water, within certain parts of the granules, which has been vaporized by the heat and confined by the external pressure. It is claimed that the porous, dry mass dissolves or disperses in cold water and forms an excellent paste or size for laundry or similar purposes. This process is similar to one that has been used for many years to produce breakfast foods from cereals, such as rice and wheat, which were described as "shot from guns." It is interesting to note that the production of an exploded starch is contemplated in a brief review given in a recent note in *Science and Appliance* (91).

7. **Modification of Starch with Gluten.** The character of a starch product or paste may be modified by the presence of various proportions of gluten or other protein. Alexander (92) has described the early use of flours, such as wheat flour, as adhesives. A considerable quantity of wheat flour of low grade is consumed in making an inexpensive adhesive for wall paper and the sealing of the seams of paper bags.

Another modification is a roll-dried product that disperses in cold water made by passing a mixture of corn starch and corn gluten over heated rolls. In one procedure use is made of the finely prepared intimate mixture of the two obtained from the wet mill houses where corn starch is manufactured, just prior to the stage at which these liquors are passed over tables or through centrifuges to separate the starch. One type of product is sold under the trade mark of Mogul (93-95). It is used for such varied purposes as an adhesive, as a core binder (mixed with sand in forming foundry moulds), and as a coagulant for certain mine wash waters.

Another manufacturer varies the process (96) by heating the suspension of starch and gluten up to about 152°F., with agitation, then filtering and drying. By the addition to such suspensions of further quantities of gluten, products are obtained that have a protein content ranging from 5 to 50%. It is claimed that the product is suitable for use in processing canned foods, since when it is used as a thickening agent the water-carrying ability of the thickener is not so extensively reduced in the cooking operation as when starch alone is used.

BIBLIOGRAPHY

1. Daly, R. E., Frankenfield, F. A., and Schopmeyer, H. H., U. S. Patent 2,373,016 (1945).
2. Schoch, T. J., U. S. Patent 2,280,723 (1942).
3. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York p 46 (1944).
4. van Hasselt, J., U. S. Patent 1,909,558 (1933).
5. Becher, W., U. S. Patent 1,910,741 (1933).
6. Staub, J., U. S. Patent 1,913,776 (1933).
7. Sjostrom, O., U. S. Patent 2,052,320 (1936).
8. Eble, O. H., and Mason M. J., U. S. Patent 2,343,048 (1944)
9. Hampel, C. A., British Patent 560,983 (1944).
10. Hampel, C. A., Can. Patent 434,496 (1946).
11. Vincent, G. P., U. S. Patent 2,409,084 (1946).
12. Schopmeyer, H. H., and Felton, G. K., U. S. Patent 2,218,221 (1940)

13. Kerr, R. W., British Patent 533,023 (1938).
14. Bulfer, A. J., *Rev. brasil. quim. São Paulo,* **17**, 272 (1944).
15. Kerr, R. W., U. S. Patent 2,052,308 (1936).
16. Fiske, A. H,. U. S. Patent 2,131,431 (1938).
17. Samec, M., Kolloidchemie der Stärke, Dresden, 275 (1927).
18. Kerr, R. W., U. S. Patent 2,108,862 (1938).
19. Bryant, A. P., U. S. Patent 1,937,543 (1933).
20. Rowland, B. W., and Bauer, J. V., U. S. Patent 2,113,034 (1938).
21. Kerr, R. W., and Schink, N. F., U. S. Patent 2,438,855 (1948).
22. Gill, L. O., and McDonald, J. W., U. S. Patent 2,407,071 (1946).
23. Traquair, J., *J. Soc. Chem. Ind.,* **28**, 188 (1909).
24. Nusslein, J., and Pauser, K., U. S. Patent 2,335,194 (1944).
25. Höppler, F., *Kolloid. Z.,* **101**, 305 (1942).
26. Maxwell, R. W., U. S. Patent 2,148,951 (1939).
27. Lolkema, J., and Kool, C. M. H., Dutch Patent 58,015 (1946).
28. Heald, A. M., *Paper Trade J.,* **113**, 39 (1941).
29. Duryea, C., U. S. Patent 675,822 (1899).
30. Richardson, W. A., Higgenbotham, R. S., and Farrow, F. D., *J. Textile Inst.,* **27**, 131 (1936).
31. Sjostrom, O., *Ind. Eng. Chem.,* **28**, 63 (1936).
32. Bloede, V. G., in Comprehensive Survey of Starch Chemistry, ed. by R. P. Walton, New York, 172 (1928).
33. Wirtschaftliche Vereinigung Deutscher Stärke-Industrien, German Patent 739,632 (1943); 6,030 (1936).
34. Harvey, E., U. S. Patient 1,726,824 (1927).
35. Pierson, G. G., U.S. Patent 2,023,973 (1935).
36. Kerr, R. W., U. S. Patent 2,268,215 (1941).
37. Meyer, K. H., Natural and Synthetic High Polymers, IV, New York (1942).
38. Biedermann, W., *Fermentforschung,* **1**, 474 (1914).
39. Biedermann, W., *Fermentforschung,* **2**, 458 (1919).
40. Biedermann, W., *Biochem. Z.,* **135**, 282 (1923).
41. Biedermann, W., *Biochem. Z.,* **137**, 35 (1923).
42. Biedermann, W., *Biochem. Z.,* **149**, 309 (1924).
43. Haehn, H., *Biochem. Z.,* **135**, 587 (1923).
44. Iljin, W. S., *Biochem. Z.,* **145**, 14 (1924).
45. Haehn, H., and Berentzen, H., *Chem. Zelle u. Gewebe* **12**, 286 (1925); *Chem. Abstracts,* **20**, 1998 (1926).
46. Zwikker, J. J. L., *Rec. trav. botan. néerland.,* **18**, 47 (1921).
47. Samec, M., Kolloidchemie der Stärke, Dresden, 8 (1927).
48. Bungenberg de Jong, H. G., *Rev. trav. chim.* **5**, 199 (1924).
49. Pierson, G. G., U.S. Patent 2,413,885 (1947).
50. Samec, M. Kolloidchemie der Stärke, Dresden, 173 (1927).
51. Meusel, E., *Jahresber. Chem.,* 2099 (1886).
52. Schaer, E., *Pharm. Centralhalle,* **37**, 540 (1896).
53. Lejeune, G., *Comt. rend.* 216, 217 (1943).
54. Caesar, G. V., U. S. Patent 2,111,204 (1938).
55. Iler, R., U. S. Patent 2,359,858 (1944).
56. Griffin, I. L., Truax, D. E., and Nuttall, N. H., U. S. Patent 2,343,898 (1944).
57. Griffin. I. L., Truax, D. E., and Nuttall, N. H., U. S. Patent 2,381,587 (1945).

58. Pacsu, E., and Mullen, J. W., U. S. Patent 2,372,337 (1945).
59. Kunze, W. G., and Evans, R. B., U. S. Patent 2,282,364 (1942).
60. Bauer, H. F., U. S. Patent 2,378,378 (1945).
61. Glarum, S. N., and Thomas, J. J., U. S. Patent 2,302,309-10 (1942).
62. Glarum, S. N., and Thomas, J. J., U. S. Patent 2,400,820 (1946).
63. Anon., *Chem. Inds.*, **62**, 88 (1948).
64. Billing, W. M., U. S. Patent 2,357,073 (1944).
65. Favor, H. H., and Johnston, N. F., Effects of Polyoxyethene stearate on Crumb Softness of Bread, Abstracts, Am. Assoc. of Cereal Chemists, Kansas City, Mo., (1947).
66. Moore, M. C., U. S. Patent 2,368,767 (1945).
67. Evans, J. W., U. S. Patent 2,400,402 (1946).
68. Kesler, C. C., and Rankin, D., U. S. Patent 2,427,562 (1947).
69. Neumann, H. T., U. S. Patent 2,417,515 (1947).
70. Young, H. H., U. S. Patent 2,422,328 (1947).
71. Gieseke, F. O., U. S. Patent 1,979,257 (1934).
72. Sichel, A., German Patent 39,150 (1937).
73. Möller, F. A., French Patent 811,598 (1937).
74. N.V.W.A. Scholten's chemische Fabrieken, Belgian Patent 445,222 (1942).
75. Kesler, C. C., U. S. Patent 2,121,502 (1938).
76. Pennick and Ford, Ltd., French Patent 819,127 (1937).
77. Bauer, H. F., U. S. Patent 2,216,179 (1940).
78. Stutzke, R. W. G., U. S. Patent 1,320,719 (1919).
79. Maier, W., U. S. Patent 1,901,109 (1933).
80. Hoppler, E. F., and Haake, J. W., U. S. Patent 1,878,852 (1932).
81. Gill, L. O., U. S. Patent 2,214,018 (1940).
82. Kantorowicz, J., U. S. Patent 785,216 (1905).
83. Kantorowicz, J., D. R. Patent 158,861 (1903).
84. Dritter, F., U. S. Patent 847,985 (1907).
85. Supf, F., U. S. Patent 918,925 (1909).
86. French, A. H., and Gill, L. O., U. S. Patent 1,985,368 (1934).
87. Pictet, A., in Comprehensive Survey of Starch Chemistry, ed. by R. P. Walton, New York, 10 (1928).
88. Anderson, A. P., U. S. Patent 707,892 (1902).
89. Anderson, A. P., U. S. Patent 769,289 (1904).
90. Anderson A. P., U. S. Patent 769,290 (1904).
91. Anon., *Science and Appliance*, **7**, 4 (1942).
92. Alexander, J., in Comprehensive Survey of Starch Chemistry, ed. by R. P. Walton, New York, 175 (1928).
93. Giesecke, F. O., U. S. Patent 1,939,973 (1933).
94. Giesecke, F. O., U. S. Patent 1,943,382 (1934).
95. Giesecke, F. O., U. S. Patent 1,974,915 (1934).
96. Walsh, J. F., and Rawlins, A. L., U. S. Patent 2,191,509 (1940).

CHAPTER IV

MANUFACTURE OF TAPIOCA AND ARROWROOT STARCHES

1. Importance of Tapioca Starch in the United States. Of the non-cereal starches, tapioca has ranked first in order of importance in the United States. As compared to the 800 millions pounds of corn starch and 20 million pounds of potato starch produced in the United States annually, for sale as such, 382 million pounds of tapioca were imported in 1939 and only about 42 million pounds of all other starches, such as arrowroot, sago, rice, and potato (1). Owing to the recent disruption in world trade, the market position of tapioca, which was apparently maintained in 1940 and the first part of 1941, thereafter declined. Whether this is a temporary condition or whether tapioca will be permanently displaced by domestic substitutes remains for the future to decide. It will depend primarily on American ingenuity in developing satisfactory starches from the waxy variety of grains (such as sorghum and maize) and from roots and tubers (such as sweet potatoes and white potatoes) at a cost low enough that our legislative bodies will be inclined to regulate the imports of tapioca starch. It may also depend on the further development of the manufacture of tapioca starch in the Americas during the present interval.

2. The Raw Material. Tapioca starch is produced from the tuberous roots of the *Manihot* plant, which is cultivated principally in the East Indies. Although the climate and soil of Java seem to be admirably suited to its growth, which conditions account for the large proportion of tapioca starch produced by the Javanese, the plant grows well in many tropical and semi-tropical countries. It was originally a native of Central America. Hence, a variety of names have been applied to the plant, depending on the locality in which it is grown to produce the starch, cassava, manioc, and yuca.

There are two general varieties of the plant, *Manihot utilissima* and *Manihot palmata.* The former contains a higher percentage of starch and, hence, is cultivated by plantations which supply the more modern and progressive mills. The manufacture of starch from this variety is complicated, however, by the fact that these tubers contain 0.01 to 0.04% of a glucoside called phaseolunatin which yields hydrocyanic acid during the milling operations, by the action of an enzyme which is present in the juices of the tuber. Unless the processes are designed to prevent it, the result is a blue-colored starch, produced as the result of a reaction between iron and hydrocyanic acid to form a ferrocyanide. Although both varieties of *Manihot* roots contain the hydrocyanic acid glucoside, the latter is concentrated principally in the outer layers of the *Manihot palmata* root and is, therefore, more readily removed by peeling the skins from the roots. The so called sweet variety is consequently used as native food and in some of the more elementary milling processes. Some botanists believe the sweet variety developed during the cultivation of the bitter. Furthermore, it is to be noted that transplantation of a given variety into a totally different climate and different soil may cause a marked change in the nature of the plant, especially in respect to the amount and distribution of the hydrocyanic acid glucoside (2).

Manihot roots cannot be stored unless they are thoroughly desiccated. The enzymes present in the roots become very active as soon as the roots are dug. In certain sections, as for example, the East Indies, where labor is cheap and abundant, the roots may be cleaned, ground, and spread out in the sun to dry and may then be used for starch manufacture at some later date. Ordinarily, however, cultivation of the roots, harvesting the crop, and milling the starch must be coordinated and this accounts for plantation ownership and operation by some of the larger millers of tapioca starch. A substitute system is for the millers to contract with independent farmers for the production and delivery of stated quantities of roots. This alternative has certain advantages that will be seen from the following considerations. The *Cassava* plant is very sensitive, among other things, to moisture conditions. It can endure neither standing water nor periods of drought. Especially during the dry periods, marked changes in the starch content of the tuber will be noted. Thus, should unfavorable conditions prevail during the season in the particular location of the plantations, the raw supply of the industry would be jeopardized, whereas if the supply is drawn from small farms, in scattered localities, assurance of a supply of roots is more certain.

In the case of small farms, however, considerable variation in the raw product may be found. Not only will the size of tubers and starch content vary, but, what is equally important, the quality of the starch exhibits variations as well. In many sections of the tropics, *Cassava* occupies much the same position as potatoes do in some parts of the temperate zones in being the principal carbohydrate of the daily diet (1). The roots may either be boiled, fried, or dried, and made into a flour for baking. Among the native peoples, there are as many individual preferences for a particular variety of *Cassava* as there are among those who rely on potatoes for daily sustenance. The native farmer has special names for most of these minor varieties but the names give no assurance of distinguishing them for starch production purposes. The names which are used to differentiate these varieties vary with locality and are usually based on some superficial characteristic of the tuber when prepared for eating.

Considerable variation in tapioca starch results also from the fact that no small share of East Indian tapioca starch originates from small mills (3) where the starch is made by crude equipment from different varieties of *Manihot* and in uncertain states of purity and virginity. This product is transported to local markets and to larger mills for further refining.

Although analysis of the tubers will show considerable variation, the figures given in Table X may be taken as average for the common varieties at harvest time. A comparison is made with the average content of common varieties of potatoes (4).

TABLE X

Composition of Manihot and Potatoes

	Manihot roots per cent	Potatoes per cent
Moisture	70.25	75.80
Starch	21.45	19.90
Sugars	5.13	0.40
Proteins	1.12	2.08
Fats	0.41	0.20
Fiber	1.11	1.10
Ash	0.54	0.92

In comparison, *Manihot utilissima,* the bitter variety which is cultivated for starch manufacture, usually averages around 30% starch at harvest time (5).

The starch granules are contained in the cells of the pith of the tuber, as they are in potatoes. However, in contrast to potatoes, the *Manihot* cells are smaller, the cell walls are thicker, and the starch granules are smaller. In both cases it is necessary to rupture the cells in order to free the starch. Any cells escaping rupture will carry their starch content to the fibrous residue, and the yield of starch produced will be decreased, proportionately. It is more difficult to fracture the cells of the *Manihot* root and hence extract all of the starch than to rupture the cells of the potato. Therefore, when highest efficiency was required, the mechanical equipment used in the older art of potato manufacture needed some elaboration when applied to the manufacture of tapioca starch on an industrial scale.

Another difference is to be noted. Although it is desirable to clean potatoes thoroughly before milling, so that separation of a white starch is facilitated, it is essential that all fine dirt be completely removed from the tapioca roots before they are processed. It is almost impossible to make a starch of prime color otherwise. This is partly due to the fact that, as the individual granules of tapioca starch separate, quite frequently a concave region develops on one face of the granule, in which depression fine particles of dirt may lodge and not be removed by simple washing. Peeling of the roots need not, however, be complete before milling.

3. Production Methods. There are two general methods for rupturing the cell walls to release the starch, biochemical action and mechanical means. In the former method, the roots are allowed to ferment or decay up to a certain stage. They are then pounded into a pulp, and the starch is washed from the pulp with water. The latter method includes breaking the cell walls by scraping, rasping, or crushing the roots.

The practice of allowing the roots partially to ferment or decay was employed by the Chinese of the Malay States during the nineteenth century and by small native millers in other sections (1). A rather complete separation of starch from fiber pulp can be accomplished by such a pretreatment, but the over-all yield of starch, nevertheless, is not materially increased, and the quality of the starch is inferior. Hence, practically all tapioca starch now produced is made by mechanical disintegration of the tuber cells.

In the most elementary case, such as the small native manufacturer who produces enough for local demands or for transport to larger mills for further refining, the miller has little else than a grater. This is usually constructed from a piece of tin, in which a number of holes have been made, the upstanding edges of these holes forming the grater. Many of the small graters of the native Javanese are constructed from a small board in which copper or iron wires are fastened to make, in effect, a metal brush. The next improvement in native production methods was the tread-grater. This consists of a hollow cylinder attached by wooden spokes to a center axle. The cover of the cylinder is made from strips of tin which are provided with teeth. A wheel attached to the cylinder is set in motion by a pedal. As the native pedals the grater into motion, he presses the *Cassava* root against the grater, while a stream of water out of a reservoir runs the pulp into a basin underneath the wheel. The grater is provided with a wooden cover to prevent loss of materials by splashing.

The starch is allowed to settle in a basin, the pulp decanted, and the settling process repeated several times with fresh water. Finally, the settled starch is allowed to stand to form a compact cake. It is then placed in bamboo baskets, in the sun, in order to dry.

The grating machine or pulper used in Javanese tapioca mills may consist of a revolving box or drum, running at high speed and covered with perforated sheet iron. As a rule a so called recrusher will be found under the grater box to complete the disintegration of the cells and insure a maximum yield of starch. Some of the larger mills employ saw-toothed rasps similar in some respects to those used in the manufacture of potato starch; *e.g.*, the Jahn Rasp. It is impossible, however, even with efficient grating devices to remove all the starch in a single operation. In order to recover some 10 or

15% of the total starch which remains with the fiber, the pulp is passed through a so called pulp mill. This consists of a special fine mesh sieve through which the pulp is rubbed and which requires a relatively large expenditure in motive power.

After leaving the grating machines, the pulp is washed with fresh water over sieves of sufficient fineness that the coarse pulp and unopened cells are removed, but not the finest pulp and starch. As a rule, two or three sieves of different meshes are used to facilitate the process.

Of the four types generally used in Java, namely rotating cylinder sieves, shakers or vibrating sieves, brush washer sieves, and fixed or stationary sieves, the first is probably the most popular. The reasons are that (*a*) various gummy materials which tend to agglutinate with the fiber continually plug the meshes of all sieves. But the rotating cylindrical sieves tend to free the meshes as the cylinder rotates. Once each cycle the sieving surface is inverted. The shakers, or fixed sieves, remain in a horizontal or slightly inclined position. They must therefore be washed frequently by stopping operations and hosing with wash water. (*b*) The shaker sieves, especially, need more repairing and are more costly to operate. (*c*) The fixed sieves are used in factories of low capacity, and where space is limited. They are, however, less efficient than either of the other types.

A few of the more progressive mills use a fourth type of sieve known as the flat brush washer. In this the pulp is discharged into the center of the sieve, where brushes gradually push it in a spiral line to the circumference. The pulp in the meantime is continually sprinkled with water from numerous sprays, so that the action of the brushes not only tends to facilitate washing the starch from the pulp but also aids in keeping the sieve holes open. The residual pulp, which comes to the edge of the sieve, is swept into a duct, by the outer brushes. An intermediate type is the cylindrical sieve with brushes.

Complete removal of the coarser fiber is essential to the production of first quality starch. Larger particles are not removed in subsequent starch settling operations. They not only contaminate the flour by their presence but also interfere with the more complete separation of fine pulp from starch when the latter is allowed to separate by gravity.

In order to separate the starch from the strained slurry, the latter is carried off to settling tanks and allowed to stand or is slowly run down inclined troughs, called tables. The first consist of cemented basins with a flexible arrangement for drawing off the supernatant liquor containing the light pulp. The latter is run into a second settling tank to recover an additional amount of starch which settles out.

The settled starch is then resuspended in fresh water and allowed to re-settle. Resuspension is usually accomplished by an agitator consisting of two wooden beams, the radius of the tank in length, which are capable of floating on top of water which is run onto the starch. They are united in a bearing which moves up and down, depending on the water level, along a central axle. Motivation is accomplished by a drag chain attached to an overhead wheel.

Settling of purified starch depends on electrolyte concentration, other factors being equal, and according to unpublished observations of the writer, the anion is more effective the higher its valence. Phosphate is quite effective. However, small additions of sulfuric acid are sometimes used in the manufacture of tapioca.

Considerable care should be exercised in controlling the final pH of the finished dry starch, which should be about pH 5 to 6. When hard or alkaline water has been used for the final purification, traces of sulfuric acid may be an added advantage in this connection also. The purest starch is made by using liberal amounts of naturally soft or softened water. Extremely hard water used in processing (high in lime) has been known to leave calcium oxalate in the finished product.

Tabling and the influence of soluble electrolyte thereon have been discussed under corn starch manufacture.

Some of the more modern factories use centrifugal separators particularly for the second or final separation. The impurities in colloidal suspension are more effectively eliminated, and the separators have the added advantage of producing a drier starch cake for the final drying operation. The moisture content in such starch cake is frequently reported to be under 40%. This content is not as low as can be obtained with corn starch when centrifugation is used in the final step but it is 10% lower than can be obtained by simple settling of starch and 5% or so lower than can be obtained by filtration. This reduction in moisture content is obviously an added advantage in speeding up the drying operation.

In the smaller factories in Java, drying the starch in the sun has been used extensively. This procedure has two advantages: the low cost and the bleaching effect exerted by the solar rays on the starch, which effect is not obtained in drying in ovens or in kilns.

However, in the more modern factories, mechanical drying is now used exclusively. The systems used vary considerably, even more than in the manufacture of corn starch in America. They vary from crude open hearths in which the starch is spread out on a galvanized iron plate over a low wood fire, and continually turned over by hand to a more or less automatic and continuous drier which is strikingly similar in some respects to the Proctor-Schwartz drier recently adopted in the United States for the manufacture of corn starch. The starch is fed automatically onto a continuous cloth belt which travels at a determined rate of speed through a drying tunnel. Instead of forced hot air drying, however, the heat is supplied by steam-heated elements underneath the conveyer belt. This method possesses three major advantages, quick drying, the small space required, and practically an elimination of biochemical activity during the drying operation.

When mechanical drying is used, chemical bleaching is frequently resorted to in the final stages of production before drying. Because of the nature of the colored impurities in tapioca starch, which are mainly extremely fine pulp particles, in contrast to the carotenoid material in cereal starch, a reducing rather than an oxidizing bleach is more effective. Bisulfite is frequently used for this purpose.

After the drying operation, the starch is sent to mills which break up the granule aggregates, and it is then bolted over sieves and packed for the market. Several grades are usually produced. There is a prime starch grade and a secondary grade, the basis of which latter is the product obtained from the second settling or retabling of the *Cassava* pulp.

The pulp residues are sometimes combined and sold as a carbohydrate feed for cattle.

4. **American Production.** Although Java has produced most of the tapioca starch of commerce, some starch has been manufactured in Europe (6) from imported, dried roots, and increasing quantities are being produced in the western hemisphere. In the latter instances greater overall plant efficiency is maintained, necessitated no doubt by higher operating costs. It should be noted, for example, that the manufacture of tapioca starch reached a surprisingly high state of efficiency in Florida in the first decade of the twentieth century (7). These mills were the first to introduce and improve on the efficient rasping devices used in the manufacture of potato starch. Even so, apparently, Florida was not able to compete with Java, owing to the wide difference in labor costs. During the last decade, however, the manufacture of tapioca starch has developed again in the Americas, particularly in Brazil (8) and in the Dominican Republic (9). Examples of American methods of manufacturing will be given to illustrate means of obtaining a high degree of overall operating efficiency.

A Brazilian plant near Cacapava receives roots in sacks by truck or rail from outlying farms. The supply is both from plantations owned and operated by the starch company and from independent farmers. The roots are dumped by hand into a receiver which feeds rotary washers capable of handling about 30 tons per 24 hours. The latter are about 3 feet in diameter and 12 feet long and are of perforated steel with horizontal openings about

$1\frac{1}{2} \times \frac{1}{2}$ inch. Inside are helical shovels, about 5 inches high, that move the roots forward and toss them. Two 2-inch water spray pipes run inside the length of the washer. Naturally soft, surface water is used throughout the process. The roots leaving the washer are clean of all dirt particles and about 80 to 90% peeled.*

At the discharge of the washer is a Jahn rasp which in turn empties into a tank. The latter feeds a coarse sieve of perforated copper. The sieve is U-shaped, about 30 inches in diameter and 12 feet long, and is stationary. A rotating shaft and paddle arms inside move the coarse pulp forward under a spray of water and finally discharge it into a second rasping machine. The washing process is then repeated on the reground pulp.

The starch liquor now goes over a series of copper shakers on each of which there are pipes that spray water to wash the starch from the finer pulp. The final starch liquor at about 3-4° Bé. is pumped to starch tables about 10 inches wide and 80 feet long, and the last of the fine pulp runs off, leaving the sedimented starch in a rather high state of purity. The starch is now made up with fresh water and sent by pump to cloth-lined basket centrifuges in order to remove the last soluble impurities as well as the fine, colloidally dispersed material. The baskets are about 3 feet in diameter by 2 feet high, with a capacity of about 130 lbs. of starch. The filling and spinning cycle is about 15 minutes on each basket.

The centrifuged starch is fed to vacuum driers about 4 feet in diameter and 16 feet long which operate at about 17 inches vacuum and 55°C. and require a 90 minute cycle to dry about 1500 lbs. of starch to about 14% moisture.

The driers discharge into an elevator to a Pope mill and bolting reel, from which the powdered starch is packed in bags.

Not only is prime starch made by such a process, the wet end of which is operated in the cold, but high recovery of starch is obtained as follows: The effluent from the tables is used to form a slurry with the washed coarse and fine pulp. This is autoclaved with a small amount of sulfuric acid, then blown into wooden tanks, and neutralized with sodium hydroxide or carbonate. It is fermented with yeast in the usual fashion. After a crude recovery, the alcohol may be refined in fractionating stills or fermented further to acetic acid, depending on the relative market price for the two commodities. The crude acetic acid is concentrated and crystallized as the sodium acetate, from which acetic acid of high strength is obtained on treatment and distillation with sulfuric acid, in the usual fashion.

---

* According to a private communication from G. Van Biema, some Brazilian plants attempt complete peeling of the roots to facilitate production of a white starch and use copper, brass or bronze equipment instead of iron where contact with wet starch mill liquors may lead to the production of ferrocyanide. Additional improvements in starch quality are secured by lowering the moisture content of wet starch cake at the centrifuges to 33-39%, vacuum drying at 25 in. and bolting the dried starch without grinding; a secondary grade is obtained by grinding and bolting the tailings.

The yield of starch averages about 20% of the weight of the roots ground. Practically the entire remainder of available starch can be accounted for in the production of the secondary products mentioned above.

One of the largest and most modern, tapioca starch factories in the western hemisphere is not far from continental United States. It is located at Quinigua, in the Dominican Republic. It was built and operated by a subsidiary of the Corn Products Refining Co. which owns plantations for growing *Manihot*. It has been estimated that this plant has exported about five million pounds of tapioca annually to the United States (1).

Both systems of maintaining a supply of roots have been used, the plantation method and the colono system. Figure 42 shows a section of the 8000 acre plantation owned by the company which operates the mill, with young plants set out in orderly rows. With cultivation, these plants grow to a height of 5 to 8 feet (although 12 feet is not uncommon) in 14 to 16 months. Cultivated plants grow large whorls of roots as shown in Figure 43. These will average 16 to 18 inches in length and about 5 inches maximum diameter. The colono system consists of contracting with small farmers to grow roots on their own farms.

Figure 44 is a picture of the Quinigua plant. Corrugated sheet iron,

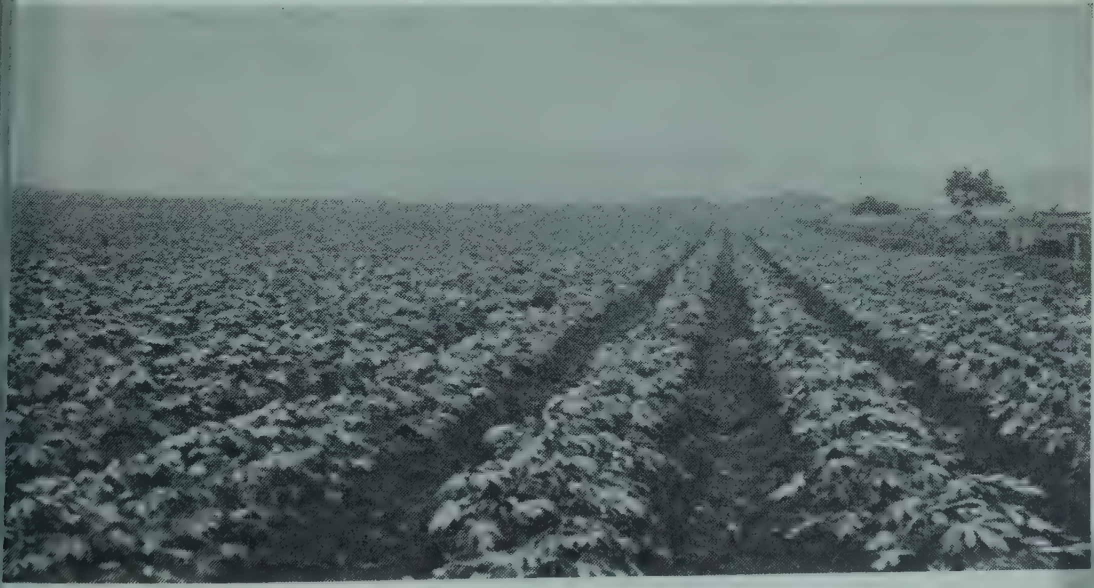


FIG. 42. Plantation of young tapioca plants

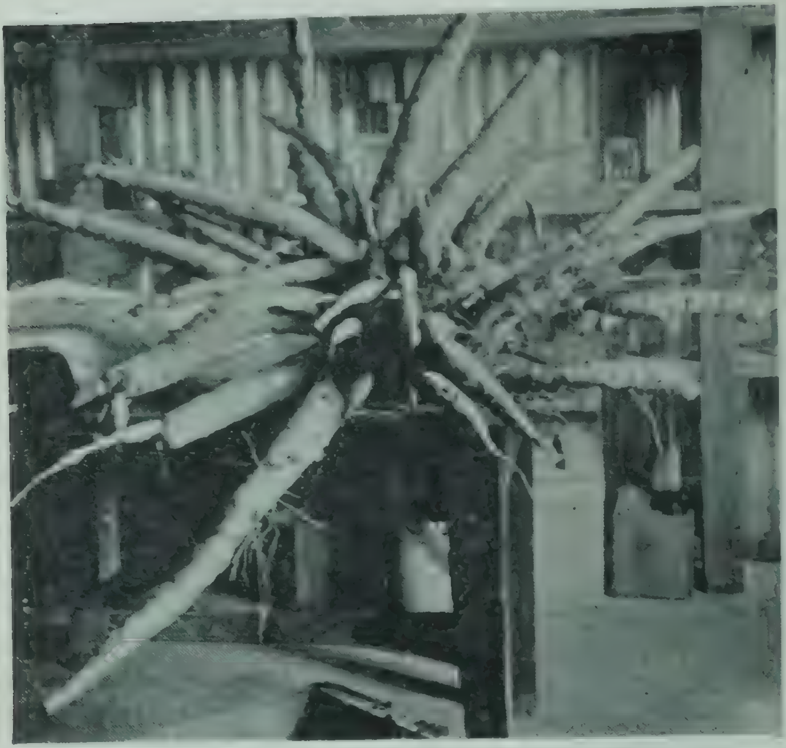


FIG. 43. Tapioca roots, showing whorl formation

FIG. 44. Tapioca starch factory at Quinigua, Dominican Republic

covered with aluminum paint is used to deflect the sun's rays and withstand tropical weather. The windows are without glass but are provided with shutters which may be closed in the event of a hurricane. An excellent yield of tapioca starch of the highest quality is produced by machinery, such as the specially designed root washer shown in Figure 45, special Jahn, saw tooth rasps, and large high speed centrifuges for final purification of the starch after tabling.

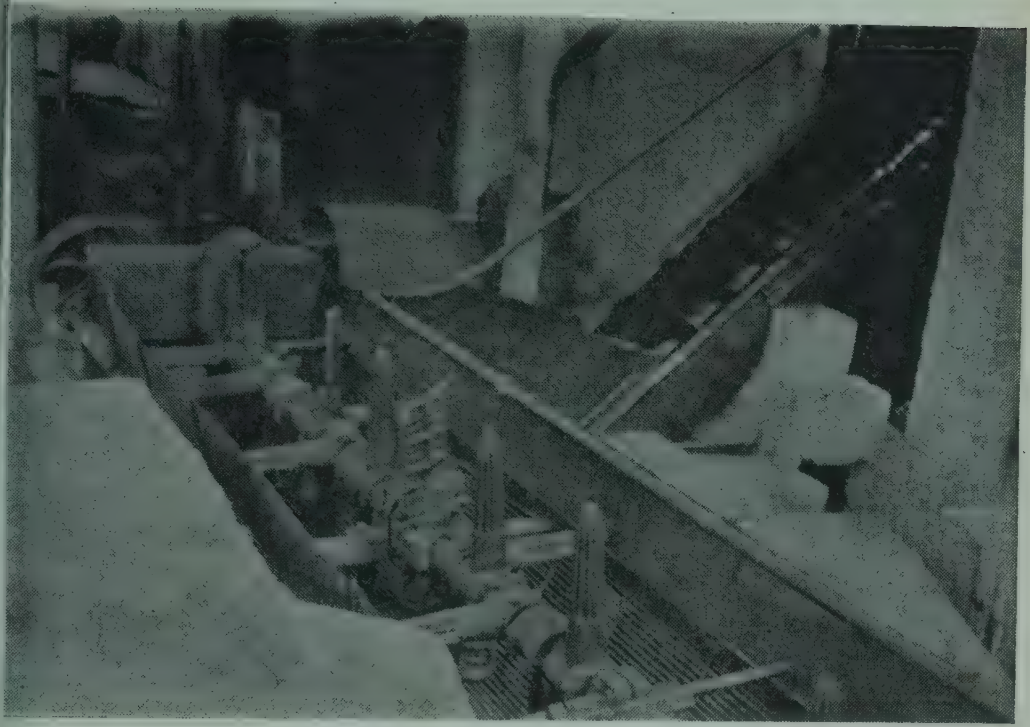


FIG. 45. Special tapioca root washers

5. Arrowroot Starch. The manufacture of arrowroot starch is quite similar to the manufacture of tapioca starch, each step in the manufacture of the latter having its counterpart in the manufacture of the former. Indeed, so similar are the two starches in respect to source, manufacture, and paste characteristics, that they are often confused and much of the arrowroot of commerce is actually a variety of tapioca. In the older literature, tapioca was often referred to as Brazilian arrowroot.

Arrowroot starch is, none the less, a distinct variety of starch, the manufacture of which varies slightly from tapioca in one or two respects, which will be discussed. The starch is obtained from the roots of the *Maranta* plant (*Maranta arundinacea*, L.). The plant belongs to the family Scitamineas, genus *Maranta*. It is a herbaceous, rhizomatous plant, having stems about a foot and a half high bearing oval lanceolate leaves, oval-shaped flowers, white in color and arranged in twin clusters, which produce small red seeds. The root, where the aerial stem starts, is elongated, somewhat flattened, and pointed. It is covered with regular scales at each section, under which scales are the germs by which the plant may be propagated (as well as from the seeds). The scales, if not completely removed, impart a bitter taste to the starch. The roots contain about 25% starch and 65 to 70% water, but due to the very tough nature of the cell walls enclosing the starch, industrial yields rarely exceed 15% except in the most efficiently operated mills.

Hence the essential differences in manufacture, compared to tapioca, are complete removal of the scales and the maceration stage, which requires the use of a series of mills with saw-like blades to tear the cells apart and hammers to convert the pulp to a paste. From the mills, the paste is passed into a reel and the starch is washed away by fine sprays of water. The washed pulp is reground at least two additional times in more efficient processes.

Even in the more progressive mills, considerable manual labor is involved in the various processes, which may account for a part, at least, of the higher market price of the starch. Equipment in the larger mills in the British West Indies is, none the less, very modern and comparatively efficient.

Drying the starch in the final stages of production is done at low temperature in driers of the Thomas heater type, in the larger mills. In these driers, endless belts carry the starch to regions of increasing temperatures, the starch finally attaining a maximum temperature of about 55-60°C. Some mills use rotary driers and some use specially designed vacuum ovens.

BIBLIOGRAPHY

1. *U.S. Tariff Comm., Rep. 138* (1940).
2. Copeland, E. B., *Philippine Agr. Rev.,* **1**, *139* (1908).
3. Handel Maatschappi Kian Gwan, The Story of Krebet, New York (1932).
4. Economic Resources of the German Colonies, *Bull. Imp. Inst.* (1913).
5. Heyne, K., Cultivo, Industrias y Commercia de la Yuca, *Rev. Agr., com. y Trab., Havana, 37* (1932).
6. Hubert, P., and Dupre, Le Manioc, Paris (1910).
7. Wiley, H. W., *U.S. Dept. Agr., Div. Chem., Bull. 58* (1900).
8. Frank, A., *Rev. brasil. chim.,* Sao Paulo, **10**, 120 (1940).
9. Colom., *J. L., Bull. Panamerican Union,* **67**, 116 (1933).

---

*The photographs shown are by courtesy of H. T. Middleton and C. R. Ridgway and the "International News" of the Corn Products Refining Co.

CHAPTER V

MANUFACTURE OF WHEAT, POTATO, AND OTHER INDUSTRIAL STARCHES

The essentials of the manufacture of other starches of industrial importance in the United States, such as wheat and potato, have been described in detail elsewhere (1-5). A brief review of these will be given, illustrating the development of the art in the United States, to supply a background for our present research on the process and the improvements that may be reported from time to time. The manufacture of other starches which are or which promise to be of industrial importance, such as sweet potato, sago, and arrowroot, has been less extensively discussed. Of the waxy starches which may be developed to replace the now less available tuber starches, the newer hybrids of waxy maize appear to be of such character that they may be milled by procedures and equipment comparable to those used in the milling of ordinary corn grains (6, 7).

1. **Wheat Starch.** Wheat starch is probably the most difficult of all starches to prepare in high yield with a reasonable degree of purity, which difficulty accounts, in part, for its relatively high market price in normal times. Complications arise in the manufacture of wheat starch owing to the tendency of the wheat gluten to become a sticky, dough-like material in the presence of small amounts of water and a smeary or slimy mass in the presence of larger amounts of water. The various processes that have been developed to separate wheat starch differ principally in the manner of conditioning the non-starchy components of the grain to facilitate their separation, particularly, the gluten.

Whole grains are used in the older processes and dry milled flour in the more recently used methods for starch separation.

The whole grains vary in analysis, depending on the variety of wheat and its moisture content. However, at an average moisture content of 14%, the following percentages may be taken as average for the other constituents: protein 12.5, fiber 2.5, ash 1.75, fat 1.65, sugar and gums 3.6, and starch 64.0. Dry milled flour usually contains less than 1% fiber, about 1% fat, 0.5% ash (unless salts have been added to the flour), and approximately 70% starch.

a. Early Methods. If whole grains are soaked in water and then pounded or otherwise crushed and worked up into a dough, it will be found possible to separate a considerable portion of the starch by transferring the plastic mass to a muslin bag and kneading the dough in a vessel containing some water. A good share of the gluten, together with the hulls, remains in the bag, whereas a part of the starch and some small particles of gluten pass out into the water. The suspension may then be stirred up and passed over a cloth filter to remove more of the gluten, whereupon the filtered starch may be recovered by allowing it to settle from the suspension and by decanting off the water. This or some modification of this method probably constitutes one of the oldest processes for preparing starch. Certainly the extraction of starch from wheat was known to the ancient Greeks and their name for starch, αμυλον (amylon), signifies that starch, in contrast to wheat flour, was produced without milling. Marcus Cato, in his "*Scriptores Rei Rusticae*," a treatise on Roman agriculture, describes a method of manufacturing wheat starch similar in essentials to the procedure outlined above.

b. Alsatian Method. It will be found, if the grains of wheat are soaked in warm water, in the experiment described, that the crushing process is facilitated and that a higher yield of better quality starch is obtained. This innovation is the basis of the so called Alsatian process. The grains are steeped in water at about 30-35°C. for 24 to 48 hours. The water is changed frequently to wash away soluble material and to prevent the development of high acidity. The grain is crushed after steeping, either between mill stones or channelled rollers. Crushing should not be so strenuous as to cause an unnecessary disintegration of the hulls. The mash is transferred to an extractor, which may consist of a finely perforated trough with rotating arms on a horizontal shaft which is used to knead the doughy mass. During this stirring process, a sprinkler pipe above the trough supplies a continuous spray of water which washes out the starch. The starch falls into a collecting trough under the perforated one.

The slurry, containing starch and some small particles of gluten, is passed over a series of shaker sieves in order to remove the gluten. After this operation, the starch is further purified by passing the slurry over starch tables or to centrifugal separators. The Alsatian process produces starch in yields of around 45%. An additional 10 to 15% of second grade starch may be obtained by reprocessing the residues from the first extraction. The final residues, consisting of hulls, wheat germs, gluten, and some residual starch are said to be suitable for use in animal feeds only. It is quite difficult to separate the gluten from the residual mass. Occasionally this is done but yields are low, seldom averaging over 5%.

A variation of the above process combines crushing with extraction. Several pairs of roller crushers, one above the other, grind the steeped grain, while water sprinklers above the rollers wash the starch away from the hulls and gluten which collect on sieves. The collected residues are usually milled a second time, so that more starch can be separated.

Second or low grade starch obtained in this process may be further purified to remove residual gluten. Agents are added which tend to swell or dissolve the gluten. For this purpose, either weak acids or alkalies may be used; *e.g.*, acetic acid or ammonia. Or the starch may be transferred to fermentation vats where, after a partial degradation or dissolution of the gluten by fermentation, the latter is more easily separated from the starch by washing and sedimentation.

c. Halle Process. In the Halle, or fermentation process, a fermentation step is employed either during steeping or directly after the steeped grains are crushed or in both steps of the process. In any event, the fermentation is prolonged, requiring about a week at 25°C. The action is induced by seeding a new mash with a portion of the sour liquors from a preceding batch. Alcoholic fermentation occurs during the early stages, then acid-producing bacteria create mixtures of acetic, lactic, and butyric acids. Finally a putrefaction sets in, producing rather offensive odors from protein degradation products. The odor is supposedly an index that the desired action on the gluten has been obtained and the fermentation is stopped lest the gluten be further transformed into a ropy or viscous mass.

After the fermentation, the sour liquors are removed, and the starch is extracted by washing. The latter is accomplished with greater ease than in processes in which fermentation is restricted. Yields of 60% of starch low in protein may be obtained by transferring the mash to a closed washing drum which rotates slowly on a horizontal axis. The outer surface of the drum is usually constructed of finely perforated metal or cloth sieves. As the drum rotates, spray water is introduced through pipes, passing through the bearings. The starch is washed out and is collected in a trough underneath the drum. It will be seen that this method is time-consuming, and the process does not permit recovery of the gluten from the wheat in a commercial form. The mills using this process often reek with ill-smelling odors, and of course losses in yield are very considerable. The sour residues are not preferred for cattle feeding. For these reasons, even though the Halle process was used almost exclusively for many years, it has now been superseded almost completely by other methods. Pure wheat gluten and products derived from wheat gluten now command such a price as to make complete recovery of the gluten very atractive. Indeed, in some instances the production of gluten has become the major objective, wheat starch assuming the position of a by-product.

d. Martin Process. One of the older methods used, which leads to the production of a gluten of high purity as well as a starch of high quality, is the so called Martin process, invented in Paris in 1835. Dry milled wheat flour is worked into a dough with about 40% of its weight of water. Dough mixers, such as are used in large bakeries, are suitable. After standing for an hour or more to hydrate the gluten, the dough is cut into large lumps. These are kneaded and rolled for an extended period of time on a grooved bed. As the dough is worked overhead jets of water slowly wash out the starch through sieves flanking the bed, to troughs which lead to starch tables or centrifuges.

Yields of gluten amount to 10 to 15%. Yields of starch of prime quality average about 55%. In addition a yield of about 20% of a starch high in protein is obtained, which may either be sold as such for certain uses or may be additionally treated to purify it.

Two companies are engaged in the manufacture of wheat starch in the United States. Both use flour as a raw material, usually a flour milled from soft wheat. Both are interested in the manufacture of derived products from gluten (8).

e. Newer Methods. Considerable interest has developed in recent years in the production of starch from cereals other than corn, and most of the work reported has pertained to improvements in methods for the production of wheat starch. MacMasters *et al.* (9) have considered the milling of oats, barley, and rye but have concluded that they are more costly sources, considering yields and milling difficulties, than corn or wheat. Hinton (10) has compared the possible by-products to be obtained from the five grains mentioned above. Morris and coworkers (11) and Bice *et al.* (12) have extended our knowledge of the composition of the wheat kernel. The latter have concluded that the characteristics of the starch from immature wheat make the latter suitable for commercial use. Shewfelt and Adams (13) have reported that soft or low protein wheat, which may not be as desirable as hard wheat for dry milling to produce flour for the baking trade, is suitable for milling to produce starch and may have the advantage of higher starch content.

Of the more recent methods reported for the production of wheat starch, several are improvements in, or extensions of the Martin process, one is an alkaline process using wheat flour, and another is a sulfurous acid process using wheat grain. Adams *et al.* (14) have described improvements in the Martin process wherein the dough formed from wheat flour and water is washed with five times its weight of water in a hemicylindrical vessel which is oscillated through an angle of 45°, 48 times per minute. Overflow from the starch settlers is recycled by returning it to the washers. Grace and Clendenning (15) have studied the effects of dough mixing time, dough conditioning, and washing temperature and time on the mechanical separation of starch and gluten. Washing time was found to be the most important variable. Callaghan and Elverum (16) have patented a process in which the dough prepared at 20 to 27°C. is washed and is worked continuously in order to cause it to cohere and to prevent disintegration during the removal of starch by the wash water. Schilling (17) has patented the use of nitrites, such as sodium nitrite, for the purpose of toughening the dough so as to minimize disintegration during the washing step and for its antiseptic action on process waters which are recycled after the removal of starch to the gluten washers. Callaghan and Schilling (18) have described the application of centrifugal separation to starch slurries obtained from the gluten extractor.

Dimler (19) has outlined a method called the "batter" process, in which wheat flour is made into a slurry with about 4 parts of water, by weight, at 40° and the resulting slurry is further disintegrated by a propeller stirrer operating at high speed. The slurry containing curds of gluten and suspended starch is screened to remove the gluten, which is then washed on the screen. The starch is recovered from the screened liquor by the use of centrifuges. Shewfelt and Adams (20) have also developed a slurry process wherein the crude gluten curds are removed on an inclined, gyrating screen of about 150 mesh. The starch is recovered in a basket type centrifuge and yields were reported of 90% starch, which contained less than 0.5% protein. Substantially complete recovery of the gluten was realized and this fraction contained about 20% starch. This starch content was reduced to about 10-12% by shredding the washed gluten and by an additional washing and screening operation. These workers used a total of about 12 parts of water to one of flour, by weight, temperatures between 25 to 30°C. and a reversing, semicircular stirring paddle to break up the wheat dough.

Dimler (19) has developed an alkaline process wherein the wheat flour is mixed with 6 parts by weight of a 0.03 N NaOH solution. This gives a dispersion with a pH value of 10.8 to 11.8, and this alkalinity is apparently sufficient to dissolve the wheat protein. The starch is separated by centrifuging the alkaline liquor and is purified by washing. The alkaline centrifugate is acidified with H_2SO_4 to pH 5.5 in order to precipitate and recover the protein. Dimler *et al.* (21) have applied this method to flours other than wheat but without as good results. From wheat, substantially all of the starch was recovered in one fraction and this had a protein content of 0.4 to 0.6%. However, included with the starch were certain alkali-insoluble solids which reduced the purity of the starch to between 94 and 98%. The recovered protein had a purity of between 70 and 90%.

The Alsatian process has been materially modified by Langford and

Slotter (22) by the use of relatively high concentrations of H_2SO_3 in the liquors used for steeping wheat grain. By this addition, the characteristic cohesiveness of wheat gluten is reduced sufficiently so as to permit wet milling of the grain and separation of the starch from mill liquors on tables by procedures quite analogous to those used in the wet processing of corn.

The wheat is steeped at 100°F. for about 24 hours with a relatively high concentration of sulfur dioxide. An SO_2 concentration of 0.3 to 0.5% is the recommended range for the steeping liquor. About 8.5 gals. of this acid are used to steep each bushel of wheat. After the wheat is steeped and the steep water drained off, the wheat is ground, preferably in a stone disintegrator of the buhr mill type rather than in the Foos mill used in the initial milling stage for corn grain. It would appear that the gluten is sufficiently "granular" in characteristics to be passed over 26 mesh copper reels, No. 17 silk shakers, and tables without causing a slime to develop on the sieves or without settling with the starch on the tables. The coarse material left on the reels and shakers is reground and again resieved. Tabling is quite satisfactory, at least with mill liquor as high as 3-4° Bé.

The gluten flowing from the ends of the tables may be recovered by settling and filter-pressing. It remains, however, in a somewhat altered physical state which may restrict its use for such purposes as those for which native wheat gluten is employed; *e.g.*, addition to wheat flour to raise the protein content of the latter above normal.

A 55 to 60% yield of starch with a protein content of 0.2% can be obtained by such a procedure on a pilot plant scale. The gluten fraction contains about 30% protein and is relatively high in starch. But further quantities of starch are readly recovered by washing the gluten after it is collected from the ends of the starch tables.

2. **White Potato Starch.** Although potato starch occupies a very important position in European markets, its importance in the United States decreased as the manufacture of corn starch was developed and extended. During the first half of the nineteenth century, a substantial portion of starch produced in the United States was made from potatoes. Even as late as 1880, there were more than 150 factories producing potato starch located throughout New England, the central, and midwestern states. A recent count showed that there are less than thirty plants, many of which are idle during some seasons. They are mostly located in Maine and are operated or owned by persons or corporations interested primarily in the sale of starch.

Most of the factories manufacturing potato starch in the United States are relatively small and are equipped with simple machinery. Since acquisition by the larger starch-producing companies, several of these have been enlarged and improved with equipment of very modern design. Several new plants have been built recently. The annual capacity of all United States mills is 40 million pounds. The average annual production is less than half of this and actually was 15 million pounds for the years 1925 to 1940.

The tubers of the potato plant (*Solanum tuberosum*) consist essentially of a brownish colored skin which surrounds a substance floury in nature when dry and which has a yellowish white color. The latter, in its natural state, however, consists of large cells which contain a high proportion of cellular fluid in which the starch granules are suspended. The external membranes of these cells are of a cellulose nature. The fluid consists of a water solution of soluble proteins, acids, and various inorganic salts such as phosphates of potassium. The average water content of the whole tuber is consequently quite high and varies between 65 and 80%. The actual starch content of the potato is, therefore, quite low, being about 10 to 25%.

The skin of the potato consists of a cellulose-like material, akin to wood in some respects. It is commonly referred to as cork substance.

The composition of the potato varies considerably, according to variety. For this reason, much work has been done in Europe to cultivate potatoes for starch manufacture. Among other things desired in a potato for this industrial use are (*a*) high yield of tubers per acre, (*b*) high starch content in the tubers, (*c*) thin skin, (*d*) low fiber content, and (*e*) low protein content. All of these factors must be balanced for the economical production of potato starch. Of course, the most important factors are the yield of potatoes per acre and the starch content of potatoes, the product of which is the possible yield of starch per acre. In general, the following may be taken as the average composition of Maine potatoes: water 70 to 80%, protein 2%, fat 0.1%, ash 1.0%, cellulose 0.5%, sugars 0.4%, and starch 10 to 30%. Organic acids are present such as succinic, tartaric, citric, and ascorbic. There are amino acids such as asparagine and tyrosine, the latter being a source of much trouble in starch factories due to its reaction with iron. Process water should be iron-free. Solanine is present during sprouting. Freezing of potatoes induces a change of some starch to sugar and soluble products, which action reduces the yield of starch. Active enzymes present in potatoes, such as tyrosinase, cause color reactions to develop during processing and thereby give an off color to the milled starch. These enzymes are oxidases; therefore modern processes are designed to keep the pulp from contact with the air or to use a reducing agent such as sulfur dioxide in the process waters. In one process, for example (23), air is excluded from the time the potatoes are pulped until the pulp is freed of water in the centrifuges.

In the United States, where potato starch constitutes but a small percentage of the total starch made, it is not economical to grow potatoes for the purpose of starch manufacture. In normal times cull potatoes are used for processing. These consist of small, broken, misshapen, or otherwise unmarketable potatoes. Those that have been frozen may also be used, but as stated above, yields are lower. In years when abnormally large crops are produced and a resulting surplus leads to unfavorable market prices, marketable grades may also be used. Up to the present, however, an average of not more than 1% of the total crop of the United States is used for starch production. Potatoes used for starch manufacture in the United States contain from 14 to 17% starch and the yield obtained by the factories averages from 10 to 12% of the weight of the potatoes. The average price paid is probably not more than ten cents per bushel.

The description of processes will be limited to more modern plants, where, under the guidance of competent engineers, 95% of the starch available is recovered (24).

Potatoes are delivered by trucks from nearby farms to hoppers which feed two washers in series. The first washer is equipped with a barrel-drum section where the cleaning action is induced by a centrifugal motion of the potatoes against the inner wall. Attached is a section in which heavy hardwood paddles force the potatoes, counter-current to a stream of water, to the exit end of the washer. They are removed from the first washer by conveyer to a second washer equipped only with paddles for moving potatoes and with water sprays for washing.

The washed potatoes pass to a hopper which feeds a Jahn, saw-tooth rasp. The rasped pulp is passed through rotary sieves, or reels, which remove the coarse particles. The coarser material is repulped. The fine pulp flows to a tank into which sulfur dioxide is introduced in the proportion of ½ lb. per ton of starch present. The gas is introduced by means of a rotameter.

The pulp is then centrifuged in a continuous, imperforate, conical bowl type centrifuge, with a spiral ribbon to remove the sedimented solids. It is called a Uhland protein-water separator. The solids are resuspended in fresh water or process water containing but little soluble matter. The centrifugate is discarded.

The suspension passes over a series of fine sieves. The remaining pulp is ground in an abrasive stone mill, after which process it passes over two shaker sieves for washing. The final pulp is discarded.

All starch milk then flows to a second Uhland protein-water separator. The solids are suspended in fresh water and pass to starch-settling tables.

The starch is flushed from the tables by jets of water at high pressure to a storage tank which feeds silk shaker sieves. The silk is about 200 mesh.

After passing these sieves, the starch is freed from water in centrifuges. The filtrate passes to the first protein-water separator. The starch cake is conveyed by a spiral conveyer to a bucket elevator which feeds the drier, after which it is pulverized in a hammer mill, bolted, and packed.

Other modern equipment in one mill includes a rotary brush sieve for the first grinding from the rasps, and a large, rotary, vacuum filter for final removal of water while the filter feeds a continuous belt drier.

The starch produced in our modernized factories compares favorably in quality with the best that has been produced in Europe.

In respect to the general manufacture of potato starch, it is interesting to note that Wiegel (25) reports that the velocity of sedimentation is increased for potato starch by the addition of an acid; *e.g.*, sulfurous acid. In a previous chapter, the effect of electrolyte concentration on the sedimentation of corn starch has been discussed. It is believed that the experiments reported by Wiegel are, mainly, another illustration of the same phenomenon and the effects are not due entirely to either hydrogen ion activity alone or to the specificity of any particular anion. That the velocity of sedimentation of starch is increased by electrolyte concentration is very easily demonstrated. If a sample of starch is repeatedly and thoroughly washed in distilled water, a suspension of it prepared and poured into two cylinders, and a few drops of a strong solution of sodium chloride stirred into one cylinder, it will be observed that practically all of the starch in the latter sample settles out at a relatively fast rate, whereas in the sample containing no salt the smaller granules, at least, settle at a materially slower rate.

In some manufacturing processes, the potato starch may be treated in addition with limited amounts of oxidizing agents such as permanganate, hypochlorite, and ozone to remove certain impurities, particularly those which impart a characteristic odor and taste to the starch. Such impurities are not necessarily objectionable when the product is used without additional modification and the odor and taste characteristics that have developed in the native starch are quite effectively corrected by the reagents mentioned to produce a satisfactory edible product. However, when the starch is dextrinized, as in the manufacture of gums that are remoistened to make adhesives for envelopes, labels, stamps, and stickers, small traces of these potato starch impurities are sufficient, with the effect of heat used in the dextrinization, to make a rather objectionable product. Even starches that have been treated with reagents are not entirely satisfactory for this purpose. The author has found that the characterisic, unpleasant taste which develops in the manufacture of remoistening gums from potato starch is prevented by pretreatment of a water suspension of potato starch with gaseous chlorine.

The operation may be performed in accordance with a method given for treating starch described by Kerr (26). This is done by introducing about 1.2 lbs. of chlorine gas per 100 gals. of starch slurry of 22° Bé. and holding it for 2 to 3 hours at room temperature after contact with the chlorine but before an antichlor is added to remove the excess of reagent. The starch is then neutralized, washed, and filtered. This pretreatment also appears to facilitate the subsequent dextrinization.

3. Sweet Potato Starch. Sweet potatoes have been used as a source of starch for over a century, particularly in Japan. Experimental work on the production of this starch was started in this country during the last part of the nineteenth century, and at least one attempt was made to produce it commercially in our southern states prior to 1934. At this time, government funds were appropriated to finance the operation of a sweet potato starch plant at Laurel, Mississippi. In addition to the primary object of aid for southern farmers and laborers, a secondary object was to supply a part of the domestic demand for root starches by extraction of starch from the roots of the sweet potato plant.

Early experimental work has been reported by Hardin (27), Shiver (28, 29), McDonnell (30), and Keitt (31). More recently the United States Bureau of Soils and the United States Bureau of Agricultural Chemistry and Engineering have carried on the research to develop economic methods of manufacture. Reference is made to the reports of Balch and Paine (32) and of Thurber (33, 34) in tracing the development of methods, and to the report of Paine and coworkers (35, 36) which summarizes production methods and other data on the subject for the Laurel, Mississippi, plant.

As a source of starch, the sweet potato (*Impomoea batata*) compares favorably with white potatoes. The starch content varies from about 14 to 28%, depending on the variety. A bushel of sweet potatoes, about 60 lbs. when harvested, yields from 10 to 12 lbs. of starch.

Except for methods used for the removal of color, the manufacturing process for sweet potatoes is quite simple and comparable to that for other root starches.

Sweet potatoes deteriorate rapidly after being harvested and must either be processed immediately or dehydrated. Dehydration by heat entails an added expense to that of a raw material of relatively high cost (about 30 cents per bushel for the grade of potatoes used for starch, as harvested, normally). Starch production has therefore been seasonal heretofore, and laborers from the farms producing the potatoes are employed for the manufacture of starch.

Potatoes from nearby farms are delivered to temporary storage bins from whence they are taken by water flumes to soaking pits. These pits contain horizontal, slowly moving spiral and potato lifter. After the adhering clay nd mud have been loosened, the potatoes pass to a rotary washer. These are otary drums about 4 feet in diameter and 20 feet long, with high pressure water sprays for removing the dirt.

The washed potatoes are transported by a bucket elevator to a hopper feeding the first pulping mill. Hammer mills are used for this operation instead of the rasps used in the manufacture of white potato starch. Here, with the addition of process water, the potatoes are coarsely pulped and passed on to a system of shaker screens, where the loose starch is removed. The pulp is then reground in a second hammer mill, and liberation of the starch is completed.

By the use of countercurrent process water, the product of the second grinding is passed first over shaker screens, where the last of the starch is washed out, then to reels, partially to remove water from the pulp, and finally to a continuous roller type press. In the latter, the rolls are forced against a filtering drum, 5 to 6 feet in diameter and 2 feet wide, by a hydraulic pressure mechanism. The pressed pulp is delivered to a rotary drier, heated counter-currently by steam heat.

The starch slurries are collected and passed over screens to remove the last of the larger fiber particles, and then are tabled. Tabling is accomplished in two operations, the last being done with fresh water. There is a 2 hour lag period between screening and tabling. This is brought about by the use of a large reserve tank which supplies the first tables. Such a lag is thought to be desirable in removing color from the starch, when the conditions of treatment to be described presently are used.

Tables are operated at a pH of about 9 (8.6 to 9.2) because of the alkaline process used. The density of the table liquors is held to about 5° Bé. The flow rate is 3 to 5 gals. per minute over 110 foot concrete tables, inclined 1/32 in. per foot. Constant feed to the tables is maintained. A constant head of starch is circulated through a header line to feed lines. The feed line consists of a ¾ inch pipe, extending vertically at least 3 feet from the header to within a few inches above the bed of the table. A quick opening valve is installed in the feed line, close to the header.

The density of the first tailing water is about 1.2° Bé., from which some starch may be recovered. The second table, tailing water is about 0.5° Bé. Starch in tailing water is recovered by use of cone bottom settlers, with an 18 hour cycle, from which the concentrate is passed to two imperforate basket centrifuges. The recovered starch is blended with starch from the first tables and passes to the second tables.

Second table starch is flushed off the tables with fresh water at 10-15° Bé., passed over a 200 mesh screen, and pumped to a tank. A 2 hour treatment was a slight excess of hypochlorite is given the starch at this point. After the treatment, the residual chlorine is eliminated with sulfur dioxide. The starch is adjusted to the desired pH and is then centrifuged free from water. The centrifuges operate at 1200 r.p.m. with a 40 inch basket. The starch leaves the centrifuges with about a 35% moisture content and is dried to 12% moisture in a batch type, vacuum drier. The dry starch is pulverized and screened over bolting silk.

The wet process is alkaline throughout. Clear, saturated calcium hydroxide solution is introduced at the shaker screens, and then travels countercurrent back to the first grind mills. Additional lime water is added at this point and if necessary, at the tables to maintain the proper pH. The process depends on a minimum of delays; otherwise microorganisms may become active, and the pH may drop off, in which case more lime water is added.

It is claimed that the function of calcium hydroxide is to solubilize certain pigments, to flocculate certain undesirable gums so that they may be removed with the pulp, and to produce a pulp of favorable consistency.

An acid (SO_2) process has been experimented with, but precipitation of impurities resulted during table separation. Moreover, excessive corrosive action was noted, which necessitated an alteration in equipment.

The control of microorganisms has been attempted with the alkaline sodium sulfite, but it is reported to give disappointing results. Frequent stoppage of the screens resulted, caused by coalescing of the sweet potato "latex," which hardened on the surfaces of the screens. The development of mold filament was noted, which interfered with sieving. Moreover, the pulp became unduly hydrated as it passed from screen to screen and finally became very difficult to free from water with a roller press. The sulfite process has been described by Thurber (33, 37). Balch and Paine (32) have described an acid sulfite process followed by an extraction of pigments with alkali. With the usual iron equipment used in milling, however, this method produced a starch colored by precipitated iron oxide. The calcium hydroxide process, used at the Laurel sweet potato starch plant, was suggested by Richee (36).

It is interesting to note that by the use of the latter process the sedimentation rate of starch is not greatly different from that of the impurities suspended in the table liquors. Hence the use of sedimentation tanks to effect a separation and possibly also certain continuous hydroseparators is not entirely satisfactory (38). Yet surprisingly enough, by the proper adjustment of the velocity of flow over suitable tables, rather satisfactory sedimentation of starch results at 5° Bé.

It is also interesting to note that the use of electrical, vibrating screens was tried in experimental runs at Laurel, but was abandoned because of an unanticipated low capacity.

With freshly dug potatoes, factory operation is limited to about 100 days, beginning the first part of September. Not more than a 3 to 5 day supply of potatoes is maintained in southern climates, owing to rapid changes that develop within the sweet potato after digging. Research has therefore been concerned with dehydration of sweet potatoes for storage and subsequent milling. The usual curing process is not feasible for preparing potatoes for storage for starch manufacture, owing to the high amylase content. Hopkins (39, 40) has found, however, that when ground or sliced potatoes are treated with SO_2 vapors, the membranes become permeable enough so that a large portion of the juice can be pressed out by mechanical means, which of course is much cheaper than total dehydration by heat. As high as 70% of the natural juice has been expressed by such a process. Flue gases are used to dehydrate the pulp to 12% moisture content, at which it is stable for storage.

It is reported (24) that an additional improvement can be made in the dehydrating process by the addition of a very small proportion of dry, hydrated lime.

The expressed juice consists of sucrose and other carbohydrates of low molecular weight and has a dry solids content of 9.5 to 11.5%. It would appear suitable as a base for certain fermentations or for concentration to a molasses.

Experiments reported in progress are a simplification in the process for the manufacture of sweet potato starch, based on the elimination of a greater part of the soluble material in the pretreatment described.

During the early part of the last decade interest in sweet potato starch was increased due principally to the curtailment of tapioca exports from the East Indies and a general shortage of starch made from other sources. Factories were reportedly built in Louisiana (24) and in Florida (41). One of the largest, with an estimated production capacity of a quarter of a million pounds daily, began operation in 1946 at Clewiston, Florida (42) and its operating processes have been described (43). Also, McNeely (44) has described the operation of the Warriner Starch Co. plant.

Boswell and coworkers (45) have compared the yields of starch from 38 varieties of sweet potatoes and have correlated yields against locality and season. Bourne and Thurber (41) have described the properties of Florida sweet potato starch and have found it suitable for the sizing of paper and textile, the manufacture of dextrins and several other uses.

However, possibly due to the seasonal variation in the supply of sweet potatoes and due to storage difficulties which have not been entirely solved, as stated above, interest in expansion in starch production has again turned to the less perishable grains.*

4. **Sorghum, Glutinous Sorghum and Glutinous Maize Starches.** In recent years, production of starch from grain sorghum, a farm crop of the plains states in America, has been extensively studied. Horan and Heider (46) have analyzed and compared a large number of varieties obtained over the region extending from Texas to Nebraska. In general, surprisingly small variations in composition of the grains were reported although some varieties contained over 80% starch. In the non-glutinous varieties, the starch showed a fairly constant ratio of amylose to amylopectin by a spectrophotometric method of analysis devised by the writer for the study of starch in whole grains. The glutinous varieties, of course, showed no amylose. Barham and coworkers (47-48) have also studied sorghum grains in some detail with particular reference to the quality of the starch. It is of interest to note that starch quality was fairly constant and did not vary with chemical composition of the grain. Some gradation in the gel strength of the starch was observed with the variety of sorghum from which it was obtained; several miscellaneous varieties had the highest gel strength, the kaffir varieties were intermediate and the glutinous varieties, naturally, the lowest. MacMasters and Hilbert (49) have compared the compositions of glutinous and non-glutinous sorghums as well as the two maize varieties. In general, the glutinous varieties were found by these workers to contain less starch and more oil and protein.

Kummerow (50) has compared the yield and properties of sorghum oil and corn oil, valuable by-products in starch manufacture. Sorghum gave a yield of about 65%, compared to corn, but contained a wax, similar in properties to carnauba wax, which could be recovered by extracting the grain with suitable solvents.

From the work of these investigators it would appear that grain sorghum can be milled by a process quite analogous to that used for corn grain, with certain changes depending on the variety of sorghum processed. For example, MacMasters and Hilbert (49) pointed out that the glutinous variety of starch is more sensitive to processing conditions. Excessive SO_2 concentrations or intensive grinding operations tend to lower the viscosity of glutinous starch more than non glutinous. Furthermore, some varieties of the sorghum grain were found to have a nucellar layer which contained pigments that imparted a color to the starch in wet milling processes. However, coloration of the starch was largely prevented by first removing the pigmented layer in a dry milling process known as pearling.

---

*Contrary to current popular reports, yields, expressed as pounds of sweet potato starch per acre per crop, compare very favorably with those from cereals, such as corn and are 1500 to 2000 pounds.

One mill, at least, has produced a sorghum flour from the glutinous varieties by a procedure involving dry milling similar to that used with wheat grain and another mill has been constructed at Corpus Christi, Texas, to process approximately 20,000 bushels per day of milo maize, a variety of sorghum grain, by a wet milling process.

Cooperative research between the U. S. Department of Agriculture, the Midwest Research Institute, some of which has been referred to above, and the Production and Research Departments of the company with which the writer is associated, has shown that milo maize may be processed on a commercial scale using essentially equipment designed and operated for the milling of corn grain. No unanticipated difficulties were encountered in milling the starch in these experimental, large scale operations. The starch and derived products were found to be quite suitable for industrial applications for which corresponding corn products are used.

Minor variations in results from those obtained with corn grain when milo maize was processed in a corn wet milling plant are as follows. Using the same draw of steepwater in gallons per bushel of grain, the water soluble solids left in the milo maize were lower. Steepwater (not incubated) boiled badly, was dark in color and contained 34.5% protein, compared to 45% for corn steepwater from the same process. There was somewhat less efficient separation of germ, coarse and fine fiber. The first led to a pressed germ of lower purity and contributed to a reduced yield of crude oil. However, the original grain which was processed contained 0.9% less oil than the average for corn. Moreover, it was found that Foos mill plates commonly used for corn were inadequate for milo maize; at least 5% of the whole kernels escaped both of the Foos mill stations. It was found, also, that the recovered gluten fraction contained 7.3% oil, compared to about 5% obtained in the case of corn. From these it is understandable that the yield of oil in preliminary runs was slightly less than 2%. In subsequent runs the Foos mills were modified with appropriate plates and improved separations and yields resulted.

Separation of starch from gluten by tabling was fair. The best separations were obtained using the same slope, or pitch of tables and the same rate of delivery to the tables as is used for corn mill liquors. The yield of starch from the one million bushels of grain processed was 59.4% and contained 0.69% protein, which average was not materially reduced by stripping in Merco centrifuges. During the last week of this run, protein in starch averaged 0.41%.

Protein in gluten averaged 41% which value was readily raised to 53% by the use of Merco centrifuges. Gluten presses operated much more effectively than with corn gluten. The yield of feed was 35.6%, which completed an overall yield of 97% for the milo maize operation.

Washing and filtration rates for the milo starch on drum type and American filters were very good and no differences could be observed between milo maize and corn starch. The characteristics of the starch obtained were evaluated when further modified by procedures outlined in Chapter III. Various grades of pearl, powdered, and lump starches, thin boiling starches, dextrins, gums and roll dried products were made on a full commercial scale. These products were tested for their several uses such as, in food products including puddings, baking goods and confections, sizing of textiles and paper products and in adhesives. In each case, the milo product was found to be quite comparable to the corresponding ones from corn. Indeed, so very nearly identical were the two types that currently no test exists to differentiate the two.

In the manufacture of sugars and syrups (see Chapter XIV), the colors of the liquors at the neutralizer station were found to be higher than for corn products but the colors of the finished products were normal for those of corn.

Hixon and coworkers (7, 51) have studied the properties of glutinous starch, particularly those of corn hybrids produced at Iowa State College, and have investigated milling procedures for processing these grains. It was found that glutinous corn hybrids could be milled by procedures very similar to those used in the wet milling of corn.

A considerable acreage of glutinous corn hybrids has been planted in recent years and at least two major starch producing companies have interested themselves in the milling of this grain for the production of starch. A report on the milling of glutinous corn in a mill used for the production of ordinary corn starch has been given by Schopmeyer *et al.* (52). Caldwell has also discussed this operation (53). Although a slightly lower yield of starch was obtained, it was in accord with the difference found by analysis for glutinous grain compared to ordinary corn grain. This difference is about 3%. These workers noted that it was necessary to run the mill starch liquors from the glutinous corn at a slower rate over the starch tables and the protein fraction settled and filtered with slightly greater difficulty. It was necessary to reduce the rate of the rotary starch driers as it was apparently more difficult to dry this type of starch.

5. Sago Starch. Sago starch is a name applied to a great variety of starches derived from the pith of various palms. Among those that have been used are *Cycas circinalis*, *Cycas revoluta*, *Sagus rumphii*, *Sagus farinifera*,

Convolvulus batatas, and *Areca oleracea.* All of the starches are similar in some respects. They are quite large individual granules which occur, naturally, in a fair state of purity. Only the most elementary equipment is needed for preparing the starch.

Considerable sago starch is made from *Cycas circinalis,* a palm fern which grows with particular luxuriance in the marshy lowlands of the East Indian archipelago. These palms grow up to 30 feet in height and 2 feet in diameter. They are cut about the sixth year of their growth, however, for starch manufacture.

The stems of the palm have comparatively thin, woody exterior, which is easily removed by splitting the stems lengthwise. The central portion, consisting of a very loose pith, is removed and cut up into small portions.

Manufacture consists of kneading these pithy particles in a current of water. The water is run over a sieve to remove extraneous pith and then into a series of troughs in which the starch settles out. The starch is rewashed, dehydrated, ground, and bolted, when it is to be sold in the form of powdered starch.

BIBLIOGRAPHY

1. Saare, O., Fabrikation der Kartoffelstärke, Berlin (1897).
2. Rehwald, F., Starch Making, London (1926).
3. Eynon, L., and Lane, J. H., Starch, Its Chemistry, Technology and Uses, Cambridge (1928).
4. Preuss, E., Comprehensive Survey of Starch Chemistry, by R. P. Walton, New York, 139 (1928).
5. Radley, J. A., Starch and Its Derivatives, London (1943).
6. Hixon, R. M., *Iowa Corn Research Inst. Pubs. Ann. Rept.*, Part II, **3**, p. 61 (1938).
7. Hixon, R. M., and Sprague, G. F., *Ind. Eng. Chem.*, **34**, 959 (1942).
8. *U. S. Tariff Comm.,* **138**, 42 (1940).
9. MacMasters, M. M., Slotter, R. L., and Jaeger, C. M., *American Miller,* **75**, No. 1, 82 (1947).
10. Hinton, J. J. C., *Biochem. J.,* **38**, 214 (1944).
11. Morris, V. H., Alexander, T L., and Pascoe, E. D., *Cereal Chem.,* **22**, 463 (1945).
12. Bice, C. W., MacMasters, M. M., and Hilbert, G. E., *Cereal Chem.,* **22**, 463 (1945).
13. Shewfelt, A. L., and Adams, G. A., *Can. J. Research,* **24**, 136 (1946).
14. Adams, G. A., Ledingham, G. A., and Grace, N. H., *Can. J. Research,* **23**, 143 (1945).
15. Grace, N. H., and Clendenning, K. A., *Can. J. Research,* **23**, 155 (1945).
16. Callaghan, R. W., and Elverum, G. W., U. S. Patent 2,388,902 (1945).
17. Schilling, R. H., U. S. Patent 2,418,669 (1943).
18. Callaghan, R. W., and Schilling, R. H., U. S. Patent 2,418,621 (1947).
19. Dimler, R. J., *U. S. Dept. Agr.*, Northern Regional Research Laboratory, *AIC-68* (1944).
20. Shewfelt, A. L., and Adams, G. A., *Can. Chem. Process Inds.,* **28**, 502, 519 (1944); *Can. J. Research,* **23**, 373 (1945).
21. Dimler, R. J., Davis, H. A., Rist, C. E., and Hilbert, G. E., *Cereal Chem.,* **21**, 430 (1944).

22. Langford, C. T., and Slotter, R. L., *Ind. Eng. Chem.*, **36**, 404 (1944); U. S. Patent 2,368,668 (1945).
23. Jahn and Company, British Patent 439,262 (1935).
24. Brautlecht, C. A., *Ind. Eng. Chem.*, **32**, 893 (1940).
25. Wiegel, E., *Z. Spiritusind.*, **58**, 205 (1935).
26. Kerr, R. W., U. S. Patent 2,108,862 (1938).
27. Hardin, M. B., *Agr. Expt. Sta., S. Carolina Ann. Rept.* (1895).
28. Shiver, F. H., *Agr. Expt. Sta. Bull., S. Carolina,* **28** (1898).
29. Shiver, F. H., *Agr. Expt. Sta. Bull., S. Carolina,* **63** (1901).
30. McDonnell, C. C., *Agr. Expt. Stat. Bull., S. Carolina* **136** (1908).
31. Keitt, T. C., *Agr. Expt. Sta. Bull., S. Carolina,* **165** (1912).
32. Balch, R. T., and Paine, H. S., *Ind. Eng. Chem.,* **23**, 1205 (1931).
33. Thurber, F. H., *Ind. Eng. Chem.,* **25**, 565 (1933).
34. Thurber, F. H., *Ind. Eng. Chem.,* **25**, 919 (1933).
35. Paine, H. S., Thurber, F. H., Balch, R. T., and Richee, W. R., *Chem. Met. Eng.,* **46**, 69 (1939).
36. Paine, H. S., Thurber, F. H., Balch, R. T., and Richee, W. R., *Ind. Eng. Chem.,* **30**, 1331 (1938).
37. Thurber, F. H., U. S. Patent 2,001,925 (1935).
38. Thurber, F. H., and Paine, H. S., *Ind. Eng. Chem.,* **26**, 567 (1934).
39. Hopkins, E. F., *Science* **87**, 71 (1938).
40. Hopkins, E. F., U. S. Patent 2,137,890 (1938).
41. Bourne, B. A., and Thurber, F. H., *Southern Power and Ind.,* **64**, 44 (1946).
42. Anon. *Chem. Met. Eng.,* **53**, 110 (1946).
43. Staff Report, *Chem. Ind.,* **58**, 416 (1946).
44. McNeely, M., *Amer. Miller,* **73**, 88 (1945).
45. Boswell, V. R., Garrison, M., Cochran, H. L., Woodard, O., Anderson, W. S., Miller, J. C., and Wright, R. E., *U. S. Dept. Agr. Circ.* **714** (1944).
46. Horan, F. E., and Heider, M. F., *Cereal Chem.,* **23**, 492 (1946).
47. Barham, H. N., Wagoner, J. A., Campbell, C. L., and Harclerode, E. H., *Kansas State Coll. Agr. Expt. Stat., Tech. Bull. No.* **61**, 47 (1946).
48. Kummerow, F. A., Barham, H. N., and King, H. H., *American Miller,* **75**, No. 1, 94 (1947).
49. MacMasters, M. M., and Hilbert, G. E., *Ind. Eng. Chem.,* **36**, 958 (1944).
50. Kummerow, F. A., *Oil & Soap,* **23**, 167 (1946).
51. Brimhall, B., and Hixon, R. M., *Ind. Eng. Chem., Anal. Ed.,* **11**, 358 (1939).
52. Schopmeyer, H. H., Felton, G. E., and Ford, C. L., *Ind. Eng. Chem.,* **35**, 1168 (1943)
53. Caldwell, C. G., *Rayon Textile Monthly,* **24**, 61 (1943).

CHAPTER VI

EVALUATION OF MODIFIED STARCHES IN PRACTICE

1. Viscosity of Hot Pastes.

a. General Considerations. The principal uses of modified starches are as a sol, paste, or gel. Therefore, the principal characteristics of a modified starch are its colloidal paste properties such as viscosity, plasticity, gel strength, rigidity, and similar characteristics. In practice, the modification of a starch is usually followed by some viscometric measurement, and the modification is carried to the point when the desired level of viscosity has been attained. After manufacture, however, the product is usually observed in supplementary tests made as a check on the perfection of the manufacturing process. The selection of supplementary tests is guided by a consideration of the use to which the starch will be put; *e.g.* by tests of the gel strength of starch used in the manufacture of gum drops, by cold paste body and plasticity measurements of starch designed for use in the clay coating of paper, and so forth.

Although the terms viscosity and fluidity of starch pastes are spoken of in industrial practice, most starch pastes do not possess vicosity in the sense in which the term is applied to the more perfect fluid bodies, such as water, but rather in that they possess an anomalous viscosity. The effect measured is usually the result of a combination of many inherent properties of starch that has been gelatinized in aqueous media. A fundamental discussion of this subject is given elsewhere in this text. In a review of methods it should be recalled that viscosity measurements are complicated principally by plasticity and by elastic effects which arise from residual structural units of the starch granules or new structural units in the paste which form through associative forces exhibited by the molecules or colloidal aggregates. The methods of measuring viscosity and the instruments employed, therefore, should be so chosen as to eliminate gross misconceptions as to the viscosity of a paste. In any event it will be appreciated that a statement of the viscosity of a starch product is rather meaningless in industrial practice without a supplementary determination which will throw some light on the additional properties of plasticity, tendency to gel, and other phenomena of the cold paste. Above all, it should constantly be borne in mind that many starch pastes are unstable in the colloidal sense and that the elements of structure which are found in these pastes are unstable. Because of this they are, in many cases, thixotropic; that is, their viscosity is abnormally depressed by increasing the applied stress.

Associative forces in starch paste are relatively slow in exerting their effects. Among other things, the presence of colloidal structures within a paste depends upon the extent to which the starch is gelatinized, the extent of dispersion, the pH and dilution of the paste, and the temperature to which it is brought to make the observation and measurement. It should be obvious, therefore, that the determination of even a relative, apparent viscosity of starch is no simple matter. To obtain reproducible results, even with suitable methods, requires the most rigid adherence to a prescribed technique for preparing the starch sample and the paste and for performing the test itself.

From a consideration of the chemical and physical properties of the starches, described elsewhere, it should be apparent that a more reliable estimate of the viscosity of starch paste would be obtained if observations were made at temperatures, pH values, and dilutions at which associative forces are at a minimum. Such conditions, however, would render the result of little practical significance. For routine testing, compromises are made in establishing the conditions of test. It is the more common practice, therefore, to make primary tests for viscosity at some dilution intermediate between those used in the industrial application and those high dilutions at which the results obtained are of academic interest only. Unless specifically stated otherwise, the pH is left in the range which is considered normal for starch. The determinations are, in general, run at elevated temperatures at which retrogradation effects are at a minimum. When it is obvious that the results so obtained would be misleading for a particular industrial use, the primary test is supplemented with a second test; for example, when the starch is to be applied as a cold, aged paste, a second determination is made after a definite cooling and aging period. It is appreciated that the latter test may be materially affected by retrogradation and gelling effects and hence is not termed viscosity but rather cold paste body.

A test which involves a more perfect measurement of viscosity has been proposed and used in practice in the past. The starch is dissolved at low concentration in dilute sodium hydroxide and the specific viscosity of the solution is observed after a definite period of time, at a fixed temperature. Very poor correlation results, however, when these viscosity values of starches from different sources, those obtained by different methods of manufacture, and starches from different sources prepared by different methods are compared with the working properties of the starch in practice. Use of the test assumes complete gelatinization and dispersion or solution. This rarely, if ever, is attained in practice. Furthermore, it will be observed that different starches (compare an acid-modified corn starch with a chlorinated starch, for example) show different rates of degeneration in alkaline solution. Quite naturally, substances which are effective buffers in alkaline media, and which are often found in starch products, will tend to give the starch an abnormally high viscosity (or low fluidity) by this method.

A complete review of the literature covering methods which have been used to evaluate a starch must, of necessity, be unduly long, because at one time or another almost every method that has been proposed to measure viscosity has been applied to starch. Such a review would be unwarranted, for many investigators reporting the use of these methods have failed to appreciate the anomalous viscosity of starches. When sound conditions for performing a particular test are developed (which is the general rule) the conditions imposed limit the practical significance of the results.

The industry has, therefore, empirically developed several procedures in which a variety of equipment is used, and these tests have apparently served their purpose admirably well. These viscosity tests fall into three main groups, as indicated above: hot paste viscosity, cold paste body, and the viscosity of an alkaline solution of the starch, the so called alkaline fluidity test.

b. Scott Test for Hot Paste Viscosity. Probably the most widely used method for determining the hot paste viscosity of starch pastes is to allow the paste to fall freely from a funnel, pipette, or other vessel with a restricting orifice. The Scott test is one of such methods. This type of equipment was first built for the petroleum industry. MacNider (1) suggested its use for starch pastes. Various modifications of the method have been introduced and are described in the literature (2).

The essentials of the method follow. A quantity of starch at known pH is stirred to a slurry with 280 cc. of distilled water in a German-silver beaker. The beaker and contents are then placed in a boiling water bath, in which, with stirring, the starch is gelatinized and heated for 15 minutes. At some definite period of time prior to 15 minutes, 200 cc. of the paste are transferred to a Scott viscosity cup, also in the boiling water bath. Usually 2 minutes are allowed for the paste to stand in the Scott cup. At the end of a total elapsed heating time of 15 minutes, the plunger valve which closes the orifice on the bottom of the cup is raised and the time in seconds is noted for a given volume of the paste to fall into a graduated cylinder. The time, in seconds required, is the specific Scott test viscosity.

This viscosity may also be considered as a relative viscosity, but not relative to water. It is relative in the sense that standard starches are used to set up permissible limits of variation in the viscosity of other starches to be tested by this method.

The same amount of water is used in making up the starch slurry for testing all starches. The actual weight of starch used is varied, depending on the range of viscosity of the particular starch to be tested. The weight of starch employed varies between 10 and 100 g. The moisture of the starch is taken into account, and the results are stated either on a dry starch basis or as the viscosity of a given weight of starch at a known moisture content whichever method is preferred for reporting the results. After the paste is tested, the pH is measured and, if it falls outside of the range anticipated, the test is repeated, the pH of the starch being corrected accordingly.

The boiling water bath should be of sufficient capacity to prevent overheating and slight variations in temperature due to convection currents and radiation. An acceptable method of heating is to blow steam at low pressure into condensate or distilled water. Multiple steam jets are evenly distributed over the bottom of the bath. An overflow and several free outlets or stacks are attached to the bath to provide a ready and continuous removal of excess steam and water. The water should boil vigorously. For comparative purposes, within a laboratory, no correction is applied for different elevations or other variables affecting the barometric pressure.

In routine testing, stirring of the starch pastes is mechanical and continuous. Smooth paddles with rounded edges, geared to a motor at constant speed, stir the paste. The stirring motion is rotational at 200 r.p.m. The paddles are deflected so that paste is moved from the outside walls of the beaker to the central axis. The beaker is then covered to prevent evaporation. In modern practice the Scott cup supplied for general viscosity measurements is modified for starch determinations. The cup is supplied with a wide aperture for overflow so that an excess of starch paste may be poured quickly into the cup. During the two minutes that the paste stands in the cup, its volume is automatically adjusted to 200 cc. The orifice of the cup is carefully adjusted, the starch used being one whose viscosity according to the Scott test is known. The latter is determined in a master cup which should be used for standardization work only. Normal, unmodified corn starch manufactured currently requires 90 to 100 seconds for the first 50 cc. of paste to pass the orifice of the Scott cup; the paste which is made up of 15 g. of starch (containing 12% moisture) and 280 cc. of added water normally has a pH of 5.0 to 5.5. Figure 46 shows a Scott test assembly and a second cup to show its overflow tube.

The amount of paste collected in the test, if preferred, may be any convenient volume. This volume should not, however, be unduly large. In any event, the head of paste in the cup will decrease in proportion to the amount of starch which is allowed to flow from the cup. The change in pressure head is reflected in a change of the rate of shear. Viscosity values of starch are closely related to rate of shear, and, unfortunately, this relationship is

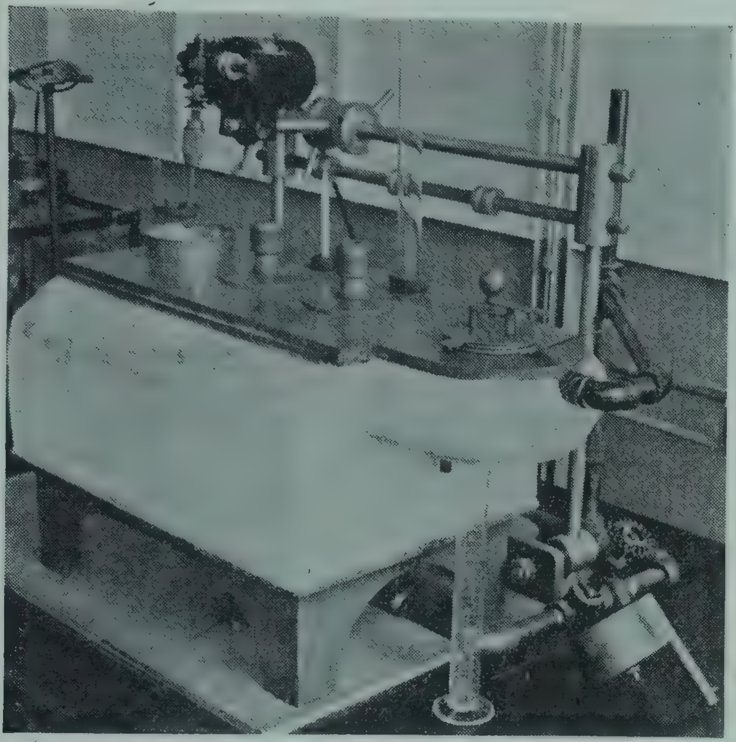


FIG. 46. Scott test apparatus for measuring the hot paste viscosity of starch. Photograph by H. Meisel.

neither linear nor necessarily the same for all types of starches. However, for comparative purposes, variation in pressure head may not be a serious disadvantage in the use of the Scott test or other instruments for measuring viscosity by transpiration methods, provided this variable is kept within restricted limits. Increasing the volume of paste collected in order to obtain significant figures for very thin starches should not be carried too far. Instead, it is the more common practice to increase the amount of thin boiling starch used in the test.

As with other tests for starch paste viscosity, absolute cleanliness of equipment is essential. A precision balance should be used, and all operations should be handled in accordance with quantitative practice.

c. Soybolt, Hot Paste Viscosity. Another instrument of the general type described above is the Soybolt, which is preferred to the Scott viscosimeter by some laboratories. The same precautions are to be observed in making a determination with the Soybolt instrument as outlined above for the Scott test.

d. Dudley, Hot Paste Viscosity. The Dudley pipette is frequently used to measure hot paste viscosities. Its use is limited, however, to comparatively thin pastes. It is, therefore, used more often on starches modified to a rather considerable degree.

The method involved is rather elementary and consists in drawing, by suction, a given volume of paste into a calibrated pipette at a definite temperature and immediately noting the number of seconds for the pipette to deliver its charge of paste into an open vessel. Normally, no provision is made for keeping the paste at constant temperature during the test, and such a procedure would, of course, rule out the use of the pipette for higher temperatures. The pipette may, of course, be jacketed for determinations at high temperature.

The method probably has its greatest use as a supplementary test in industrial modifications of starch. It may be used to follow the enzymic liquefaction of a starch in practice, *e.g.*, the action of diastase up to 70°C., and to check the anticipated extent of such liquefaction before application of the product at some elevated temperature, *e.g.*, 50°C.

Dudley pipettes are calibrated, water being used as a standard.

e. Other Tests for Hot Paste Viscosity. Other types of similar equipment either of special design or standard equipment are also used. The Redwood viscosimeter appears to have been used in England (3), whereas the Engler (4) is extensively used in Germany. It might be remarked at this point that the United States Bureau of Engraving and Printing specifications for certain modifications of starch specify viscosity by Engler.

Another modification used in supplementary tests should be mentioned. This involves determining the viscosity, by any suitable method, of a paste prepared by cooking starch with open steam jets. It is common practice in the industrial application of starch to prepare the paste with live steam as a source of heat rather than by an indirect application of heat such as a jacketed kettle. As might be anticipated from a consideration of the fragile nature of starch, the viscosity of a starch paste, as well as other colloidal characteristics which are a function of the extent to which a starch is dispergated, may be quite different when the paste is prepared by being brought to and held at a given temperature with live steam than when a similarly made paste is prepared by indirect heating. When this modification in cooking is used, care should be exercised that proper agitation or stirring is provided either by the proper arrangement of the steam jets in the cooking vessel or by supplying additional mechanical stirring. Obviously the steam used should be dried and corrections made for the steam condensation, for the evaporation of moisture during the cooking period, or for both.

2. Cold Paste Body.

a. Stormer, Cold Paste Body. When an evaluation of cold paste body is desired, the Stormer viscosimeter may be used to good advantage. Alsberg and Rask (5), Kerr (6) and others have used this type of instrument. It is comprised of a cylinder immersed in the test paste which is contained in a metal cup surrounded by a water bath. The immersed cylinder is rotated by a free-falling weight acting through a gear and pulley system. The time in seconds necessary for a given weight to produce a certain number of revolutions is taken as a measure of cold paste body. A revolution counter is a part of the instrument.

Details for applying the method follow. The starch is gelatinized in the same manner as recommended for performing the Scott viscosity test. Heating is continued for the full 15 minute period without transfer of the paste to the Scott viscosity cup. Instead, the paste is placed immediately in a constant temperature water bath, preferably 25°C., in a closed container. At the end of a definite aging period, any surface skin on the paste is carefully removed and discarded. The remaining paste is very gently stirred with a spatula for several seconds, whereupon it is transferred to the cup of the Stormer viscosimeter, and the viscosity is noted at 25°C.

The weight of starch used in making up the paste should vary in a fixed manner with the range of cold paste body of the starch. For unmodified corn starch, 10 g. plus 280 cc. of water make a suitable ratio. For the thinner, acid-modified starches, a ratio of 15 g. to 280 cc. of water is recommended. In any event the dilution should not be so high that the results lose practical significance. On the other hand, the concentration of starch should not be so high that the paste will gel when cooled. Even for rather plastic pastes, incorrect results may be obtained because of the tendency of the cylinder to cut through "short" pastes or to wind up viscid pastes around the shaft of the rotating cylinder.

Cold paste body depends upon the extent of gelatinization, the degree of dispersion, the temperature and length of heating, the speed of cooling, the minimum temperature to which the paste is cooled, the length of the aging period at a definite temperature, agitation during cooling or aging, pH of the paste, and other variables. All operations for preparing the final paste for tests must, therefore, be most carefully standardized. In routine tests, observations are made after 3 hours aging at 25°C. and occasionally on a duplicate sample after 24 hours.

Each type of modified starch requires adjustment for the weight used to actuate the cylinder in the Stormer viscosimeter and the number of revolutions counted. If too heavy a weight is used, so that the speed of the cylinder is too fast, an error results from turbulence in the paste. If the weight is too great and the number of revolutions counted is excessive, the result is complicated by the thixotropic nature of starch pastes. In most cases the first revolutions counted will be found to require a substantially longer time than the later revolutions. Although this effect is difficult to eliminate completely, it should not be exaggerated. If the weight used is too small, and particularly if a great many revolutions are counted, the reverse effect may be found. The paste may appear to become thicker owing to retrogradation or gelling tendencies exerting their influence during the period of test. A balance between the two opposing effects would, of course, represent ideal conditions. For unmodified corn starch pastes, made as described above, a 75 g. weight is used, and the time in seconds for 50 revolutions is taken as the Stormer test for cold paste body. For the medium range of acid-modified starches, 15 g. of starch are used in the test and a 50 g. weight and 50 revolutions are satisfactory.

b. MacMichael Viscosimeter Tests. The instrument introduced by MacMichael (7) has been applied to the determination of starch viscosity by Gallay and Bell (8) and others. The instrument may be used for cold paste body. This instrument consists of a cylindrical cup, in which the starch paste is placed, surrounded by a bath with an electric heating device to regulate the temperature. The cup and bath rotate at a constant speed which may, however, be regulated over a wide working range. A second cylinder is suspended in the paste by means of a torsion wire. The twist that develops in the torsion wire is read by noting the position of a fixed pointer in relation to a moving scale mounted on the torsion wire. The instrument is supplied with several interchangeable torsion wires so as to permit measurements over a wide range of viscosities.

Owing to the constantly applied shearing stress throughout the determination, most starch pastes exhibit a constantly falling viscosity. Especially for unmodified corn starch, one is in doubt as to how long to wait before making the observation or whether to take a reading the instant the motor starts rotating the cup. The latter is obviously too high in that the plasticity factor is overemphasized. Yet, if an interval is allowed before an observation is taken, one cannot be sure that other starches will show the same rate of decrease in the apparent viscosity or cold paste body.

The instrument is applicable, however, in a limited way, to certain classes of modified starches. It is particularly useful in certain supplementary viscosity tests, mentioned at the beginning of this chapter. These tests involve application of the starch in a manner similar to that used in practice; *e.g.*, starch may be used to coat papers with clay in the manufacture of printing paper of high grade. The starch, therefore, is gelatinized and mixed with the clay in a definite manner comparable to that used in practice, and the viscosity of the mixture is determined in the MacMichael viscosimeter at 5°C. Various and significant differences will be observed in the colloidal behavior of the several types of modified starches tested by such a procedure. For such tests, the viscosity in degrees MacMichael may be observed at several different speeds of rotation. These values are plotted and, if the function is essentially linear, a specific plasticity, as well as the viscosity, may be determined from the data obtained. The plasticity is proportional to the value at which the extrapolated curve for viscosity intercepts the axis of the degrees MacMichael.

c. *Hoeppler Viscosimeter for Viscosity Measurements.* An instrument which involves the use of a different principle from those described above is the Höppler (9). A recent discussion of this instrument is given by Wobser and Müller (10). Measurements are made of the time required for a given weight to fall, in vertical motion, through a measured column of paste. The column is surrounded by a constant temperature bath so that with only slight modifications the instrument can be adapted to the measurement of hot paste viscosity as well as cold paste body. However, the instrument would seem to be more adapted to the precise measurement of the cold paste body of modified starches.

Various weights calibrated so that with fluid bodies the viscosity in centipoises may be readily estimated from the time required for the weight to fall through the column are supplied. The weights are in the shape of spheres and are of such magnitude that a wide range of viscosities may be covered.

Pastes may be prepared by a standard procedure such as those outlined for the Scott and Stormer tests. As soon as it is prepared, the paste is transferred to the glass tube of the instrument. The sphere, selected from the six supplied by the maker, is placed on the tube after the tube is filled with the paste. A definite period is allowed for the paste to come to and stand at the temperature at which the observation is to be made. Constant temperature is maintained in the instrument by circulating water from a large constant temperature bath through a glass jacket surrounding the column of paste.

At the time of observation, the sphere is caused to fall through the paste several times by inverting the tube and attached water jacket, which are attached to a stand by a swivel joint. Finally, a 3-minute interval is allowed and the tube is again inverted and the time required for the sphere to fall from the upper mark on the column to the lower mark is noted. Several observations are made and the results averaged. Three-minute intervals are allowed between successive observations.

The choice of the sphere to be used is governed by the rate of fall, which should not exceed 1 cm. per second. Valenta (11) and others have pointed out the danger of the sphere not following a straight, centric path in instruments of this type. Höppler has inclined the tube to an angle of 10° in his viscosimeter. This is claimed to eliminate the tendency of the sphere to follow a spiral path in its fall. Constancy of the angle of inclination is assured by leveling the base and stand of the instrument with the aid of a built-in spirit level. Although the column may be rotated around an axis in order to reverse the direction of motion of the sphere, a set-screw is provided by which the position of the column can be fixed at the correct angle.

In instruments of this type it is essential that the sphere should be released under the surface of the test liquid and should be accompanied by a minimum of vibrational disturbances. The design of the Höppler instrument has reduced the end effects of the column to minimal proportions. The operator must exercise his ingenuity to reduce the effects of vibrational disturbance which may result from uneven temperatures. Too rapid cooling of the paste and running the test at too short an interval after placing the paste in the tube are sources of such error. After several preliminary inversions of the tube, it is essential that a sufficient interval be allowed for the movement of the contents to subside. A period of 3 minutes is recommended for starch pastes. However, pastes from certain starches may require a longer period of rest to eliminate erratic results. In inverting the column care should be taken to avoid undue vibrational disturbances.

Some difficulty may be experienced with highly opaque pastes in observing the fall of the sphere.

It will be seen that there are certain obvious advantages in using an instrument of this type, *e.g.*, simplicity of operation, speed of setting up equipment and in determination of the results, possibility of repeating determinations on the same sample, reduction of the personal factor to a minimum, and ease of standardization.

3. Pasting Characteristics of Starches or Paste History.

a. Caesar Consistometer. Certain viscosimeters may be used to measure several characteristics of starch simultaneously. The starch slurry is introduced into the apparatus, heat and agitation applied, and a record is obtained of the body of the starch over the entire period from the time gelatinization starts until the paste is cooled to a given temperature. The use of such instruments is becoming more of a practice since Caesar (12) directed attention to the value of obtaining a continuous history of the pasting operations. Such characteristics may be evaluated as (*a*) gelatinization point of the starch, (*b*) peak viscosity obtainable with a definite cooking procedure, (*c*) specific viscosity after a prescribed cooking operation, (*d*) rate of increase in paste body with falling temperature, (*e*) cold paste body after a time interval at a lower temperature. All of these characteristics are important to know when starch products are applied in industry.

The essentials of the method described by Caesar are to suspend a standard paddle, connected by a shaft to an electric motor, in a beaker containing the starch slurry. The latter is surrounded by a heating bath, and the temperature is raised at a regulated rate. After the starch is cooked, cold water may be introduced into the bath to cool the paste. The paddle and motor move at constant speeds. Changes in the viscosity or paste body are indicated by the differences in the electrical input to the motor to maintain a constant speed of stirring. To obtain significant differences in observed values, however, it is recommended that a relatively high ratio of starch to water be used in the test, for example, 20% starch. Radley (13) has used a similar type of instrument, except that the power input to the motor is kept constant and a variation in the speed is recorded.

1. Brookfield Viscosimeter—A commercial instrument known as the Brookfield viscosimeter is built on a slightly different principle. Spindles of various types are driven by a synchronous motor and the "drag" of the starch paste, acting on the spindle, is taken as a measure of the viscosity.

2. Brabender Amylograph—A more recent improvement in an instrument in which the same general principle as outlined above is used is the Brabender Amylograph. A lengthy discussion of this comparatively complicated instrument is given by Müller (14). In this method, the paste is held in a cup surrounded by an air bath for temperature control. The cup is revolved at constant speed. The measuring device consists of a disc to which are attached several short rods extending into the paste. This mechanism serves as a stirring unit also. The torque impressed on the measuring unit is transmitted to a recording torsion balance. A continuous, graphical record is traced by the instrument over the entire period of test. An elaborate temperature control is provided to increase the temperature of the starch at a fixed rate. The rise in temperature may be checked at any point and held at this level by a thermostatic control. Then, by switching off the heat supply, the paste may be cooled to any desired temperature and the change in viscosity noted Figure 47 illustrates the Amylograph.

In addition to obtaining a permanent pasting record of a starch, such characteristics of a starch may be estimated from the graph as (*a*) gelatinization point, (*b*) rate of gelatinization, (*c*) peak viscosity, (*d*) viscosity after any specified heating period, and (*c*) congealing properties such as the rate and amount of increase in the viscosity with decrease in temperature.

The instrument appears suited for other supplementary tests. The manufacturer suggests that it may be used to study the liquefaction of starch by enzymes. It may also be used to study the congealing tendencies of starch

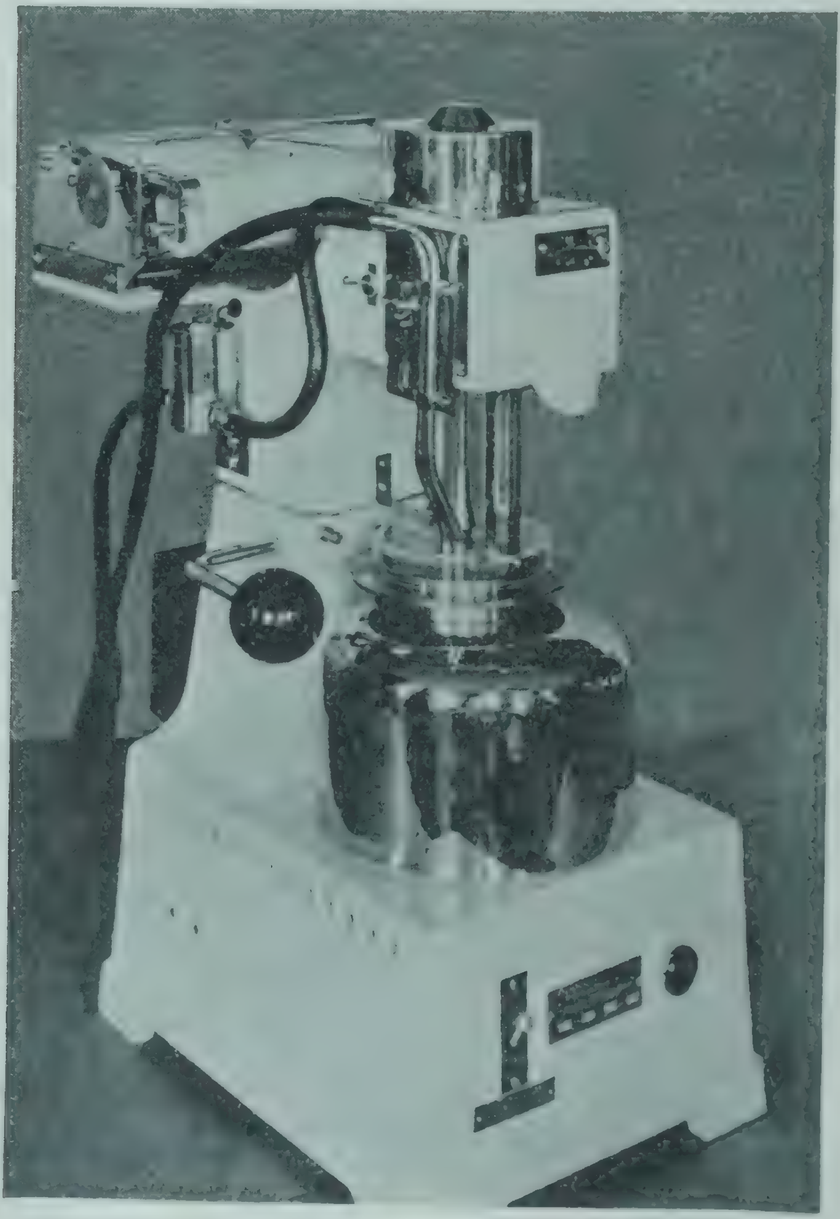


FIG. 47. Brabender Amylograph.

roducts precooked by procedures identical to those used in industrial ractice such as prepared adhesives and sizes.

Several springs are supplied with the Amylograph. By a proper choice of springs, a wide range in viscosities may be measured. Specific instructions are supplied by the maker for operating the instrument.

In application of the instrument to obtain a "paste history" of starch a slurry is made up of 35 g. of dry starch and 450 cc. of water. With a lighter spring adjustment this concentration is suitable for the accurate evaluation of unmodified starches such as corn starch. This concentration may also be used for a comparison of the pasting properties of modified starches, such as acid-modified corn starches of 20 and 40 fluidity, with unmodified starch. However, for the more accurate evaluation of modified starches and comparison with other modifications, a starch ratio of 55 g. of starch to 450 cc. of water is suggested.

The cup containing the starch slurry is set in motion, and the electric heating unit is turned on. While the temperature of the starch is raised at a uniform rate, the curve drawn by the instrument is plotted in terms of time units against viscosity units. It is desirable therefore to calibrate the curve during the period of heating and cooling, in terms of temperature. This is done manually, by watching the thermometer in the paste. At every 5°C. change in temperature (or more frequently, if desired), a mark is made on the curve by quickly moving the tracing pen through a small arc, which movement causes the pen to make a mark as the spring draws it back into position.

After a temperature of 95°C. is reached, the control is thrown over to the constant temperature setting, and changes in viscosity are noted. Viscosities normally fall off slowly. If the period of heating at 95°C. is extended beyond 15 to 20 minutes, the results become complicated by losses of moisture.

At the end of the period of cooking, the heat is turned off, and increases in viscosity for an agitated paste are noted as the temperature gradually decreases. A cooling coil is provided for the circulation of cold water and this may be used to accelerate cooling of the cooked paste when it is desired to measure consistency changes due to a reduction in temperature within a short period of time.

Anker and Geddes (15) have made an extensive study of the pasting characteristics of various starches and modified starches as shown by the Amylograph. Pasting curves were obtained, the shapes of which were found to be characteristic for the different starches studied. The effect of a number of variables on the pasting process was reported and it is interesting to note that for both corn, potato, and wheat starches, a straight line was obtained when the logarithm of maximal, or peak paste viscosity was plotted against the logarithm of the concentration of the starch used.

A rotating cylinder viscosimeter has been described by Barham *et al.* (16) which provides for reading the paste consistency during the pasting process.

During recent years, an extensive study of the pasting characteristics of starches, and of methods for measuring these characteristics have been made under a fellowship established by the Corn Industries Research Foundation. This study has led to the development of the Corn Industries Viscometer which has been described by Kesler and Bechtel (17). The instrument, shown in Fig. 48, is provided with a stirring device of unique design, which

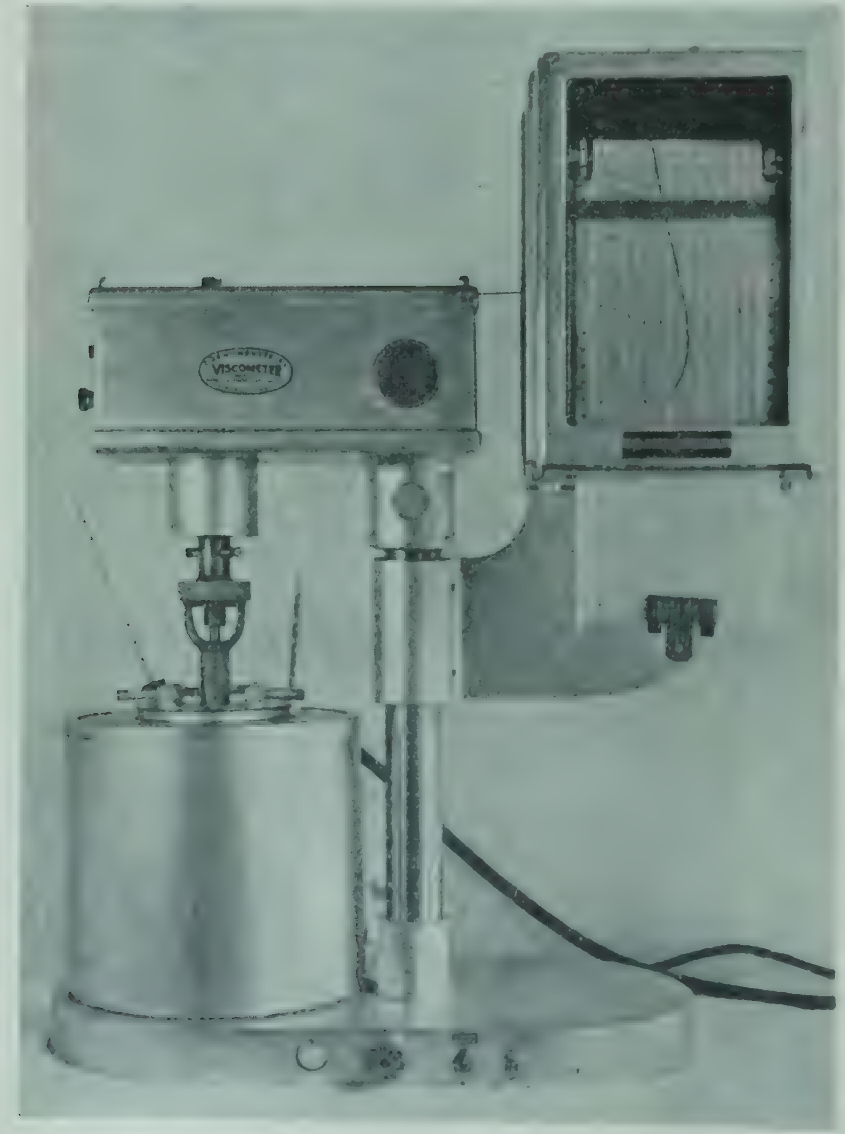


FIG. 48. Corn Industries Viscometer

erves to remove the layer of pasted starch from the walls of the vessel which s heated by a thermostatically controlled bath. A separately mounted propeller, driven through a gear differential, stirs the center of the paste and also serves as a means of measuring the viscosity changes. The force which the propeller encounters is continuously balanced by a dynamometer, consisting of a weighted arm which moves through a vertical arc. A series of interchangeable weights are provided for the dynamometer so that it covers a relatively wide range of torques between 250 and 2,000 gram-centimeters. A pen, which is attached to the dynamometer, continuously traces the record of viscosity changes on a strip type recorder. The operation of this instrument may be readily seen by reference to Fig. 49. The cover for the vessel holding the starch is water cooled and acts as a condenser, thus eliminating evaporation of water from the paste during the heating period.

Bechtel (18) has shown the results obtained for various starches and modified starch products with this instrument and has shown its suitability for research and for the control of modification of starches. For the latter, a relatively rapid procedure has been given in which the heating bath is brought to operating temperature, usually between 92 and 96°C., before introduction of the starch slurry into the pasting cup. It is pointed out, however, that a rapid rate of heating the starch increases the maximum viscosity to a marked extent, compared to that obtained with less rapid heating, and that the increase in viscosity, so obtained, remains for the balance of the test. A very great acceleration in the fall of viscosity from the maximal value was found to result from relatively small changes in cooking temperature over the range 92 to 96°C. Pasting characteristics of any starch were found to be significantly altered by changes in the concentration of starch: an increase in concentration results in an initial rise in viscosity at a lower temperature, a more rapid rise in viscosity, and a more extensive breakdown in the body of the paste from its maximum. The linear relationship between the logarithm of maximum viscosity and logarithm of starch concentration, found by Anker and Geddes (15) for corn, potato, and wheat starches was also found to hold for tapioca and acid modified corn starches.

Bechtel and Kesler (19) have shown the utility of this instrument in a study of the application of various starches for industrial use. It was reported that by obtaining full knowledge of the effect of various factors in the cooking procedure and by an appreciation of the changes which result in pastes on cooling, a more perfect correlation results between such laboratory testing and mill practice, and accordingly, the applicability of starch products for specific industrial uses may be determined with greater precision.

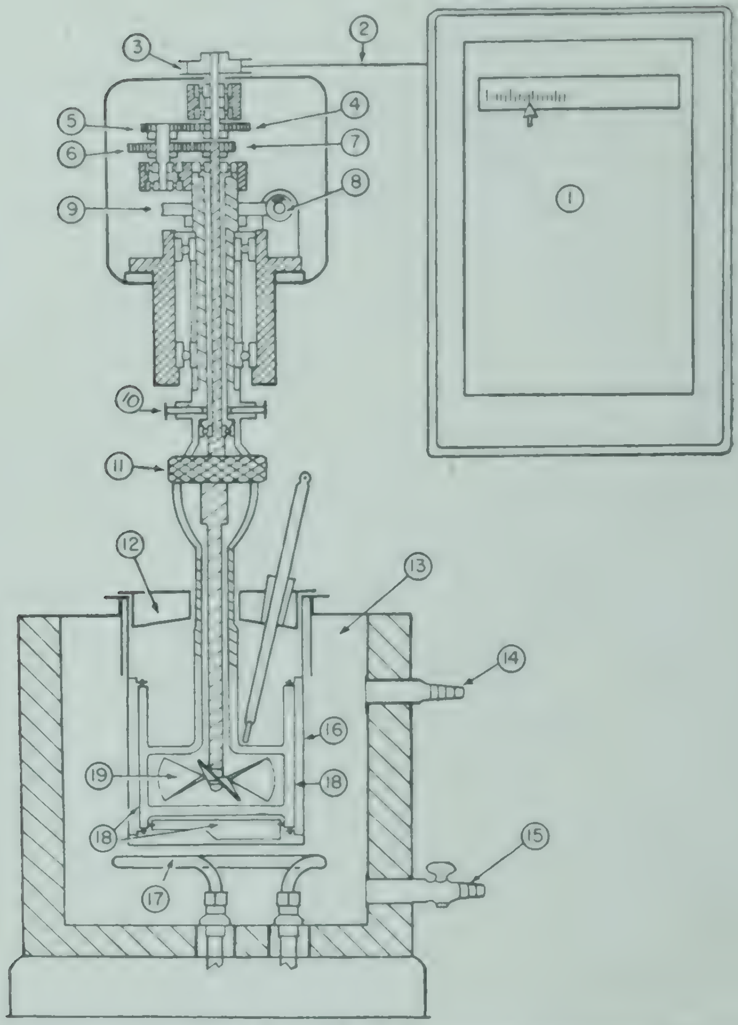


FIG. 49. Sectional drawing of the Corn Industries viscometer. (Courtesy of Welker Bechtel)

1. Recorder and dynamometer. (Dynamometer not shown).
2. Cable from viscometer to recorder.
3. Cable drum.
4, 5, 6, 7. Gears of sun and planet differential.
8. Worm, turned by synchronous motor (not shown).
9. Worm gear.
10. Spring pins for holding center shaft.
11. Coupling to attach stirrer.
12. Condenser and cover.
13. Water bath.
14. Overflow.
15. Drain cock.
16. Starch beaker.
17. Electric heater, thermostatically controlled.
18. Scraper blades.
19. Propeller.

Many laboratories of starch producing companies in the United States have provided themselves with this instrument as a means for more uniform standardization of starch products throughout the industry.

Higginbotham (20) has described a consistometer which is similar in principle to the Corn Industries Viscometer. This worker prefers, and has shown the advantages for a slightly higher operating temperature, usually 99°C. The characteristic viscosity curves obtained for various native and modified starches with this instrument and the effect of variables under these operating conditions have been very ably reported.

4. **Alkaline Fluidity.** Although the use of this test for evaluation of a starch has fallen into disrepute in some laboratories for considerations already given, a description of the method will be given for two reasons: First because the well-known acid-modified starches were originally graded according to a fluidity scale by this method, and these ranges of modification by acid are still comparable to those used heretofore; secondly, the test is still employed by some industrial laboratories for controlling the manufacture of certain modified starches. In the latter case, when the complete history of a modified starch is known, the use of the test for this purpose is perfectly justifiable.

Five grams of starch, dry weight basis, are wet with 10 cc. of distilled water in a Pyrex beaker. At 25°C., 90 cc. of a 1% solution of sodium hydroxide are added, with stirring, and the stirring is continued for 3 minutes from the time the sodium hydroxide is poured in. The mixture is allowed to stand 27 minutes more at 25°C. At this time the contents are poured into a standardized glass funnel with a special tip and the quantity of starch solution which runs from the funnel in 70 seconds at 25°C. is noted. This amount, measured in cubic centimeters, is taken as the fluidity; *e.g.*, the acid-modified, thin boiling starches of commerce with a fluidity of 40 will be fluid enough for 40 cc. of solution to pass the funnel tip in 70 seconds, with a fluidity of 60, 60 cc., etc. If 100 cc. of water are poured into the standard funnel at 25°C., 100 cc. will be delivered in 70 seconds. Relatively then, water might be said to have a fluidity of 100 by this test.

Alkaline solutions of unmodified corn starch will hardly run through the tip of the funnel. Their fluidity is of the order of 1. In some laboratories where the alkaline fluidity of the thicker starches is compared it is customary to reduce the amount of starch used in the test to 2 g. or less.

When alkaline fluidities are no longer used to appraise the fluidity of modified starches, Kerr has proposed (6) that the fluidity may be indirectly determined by use of the Scott viscosity value, the determination of which has been given above, in the following equation.

$$\text{Fluidity} = \frac{2000}{\text{Scott test value}}$$

This particular Scott test is run with 28.35 g. of starch to 280 cc. of water. For example, if the Scott test of a thin boiling starch is 50 then it is a starch with a fluidity of 40.

The use of alkaline gelatinization of starch to prepare a paste for viscosity measurement has been practiced for many years by starch chemists. It was not until 1912, however, that the method was introduced to the corn starch industry by Buel (21) in substantially the form outlined above.

Certain oxidized starches (*e.g.*, the hypochlorite-treated corn starches of commerce), if free from buffers, give results by the alkaline fluidity test which are not related to the Scott test according to the equation above. The determined fluidity is much higher (viscosity lower) than that estimated from the Scott test. Indeed so characteristic is this discrepancy that it may be used as an aid in the identification of an oxidized starch. Supplementary chemical tests may then be used to establish the type of oxidant used in the manufacture of the unknown sample.

5. Gel Properties.

a. Gel Strength. The gel strength of starch pastes has been measured by a variety of instruments embodying different principles. As might be expected, different characteristics in a gel may be measured by these tests, although the strength of the gel may or may not, depending on the viewpoint. Several of the instruments employed in practice are the rigidometer, penetrometer, Tarr-Baker jelly tester, and similar instruments. The rigidometer measures the rigidity of a gel, or its lack of elasticity, the Tarr-Baker (and instruments of that type) gives a value which is proportional to the force necessary to rupture the gel and which is also probably a function of the elastic limit. The results of tests with the blunt plunger type penetrometer are a combination of plastic and elastic effects. The tube type of plunger measures the resistance of the gel to a cutting action. The values so obtained need not necessarily show a correlation. Certain types of rubbery gels show high elasticity but fracture at values less than those required to break the structure of sort, firm gels. Plastic gels such as those exemplified by modified tuber starches possess a low elastic limit, yet give a high reading by some of the penetrometer type of instruments. All of these varied characteristics are of importance, however, in considering specific industrial applications of starches.

b. Tarr-Baker Jelly Tester. Gel strength is conveniently determined by the Tarr-Baker jelly tester. This instrument was described by Baker (22) and modified by Tarr (23). In principle, a pressure is gradually applied to a plunger, of known area, resting upon the surface of the gel. The pressure is read at the instant that the gel breaks.

The directions are as follows: Weigh carefully 8 g. of unmodified corn starch or 12 g. of acid-modified, thin boiling corn starch, and other starches in proportion to their relative viscosity. Transfer to a porcelain cup, insert a stirring rod, and weigh. Add 100 g. of water. Cook in a boiling water bath for exactly 30 minutes, timing from the instant the sample is put in the bath. Stir the sample during the first 5 minutes, and then cover with a rubber stopper through which a hole is bored for the stirring rod. Stir for a few seconds at the end of 10, 20, and 30 minutes. Cool quickly with occasional stirring and add sufficient water to compensate for moisture evaporation. After mixing in the added water, if the latter is necessary, fill two aluminum moisture dishes, 55 mm. in diameter by 17 mm. in height. Cover at once with a film of light mineral oil. Place in a water bath at 20°C. for 1 hour.

Drain off the oil and place the dish under the plunger of the tester. With 500 cc. of water in the Tarr-Baker jelly testing jar at the start, adjust the flow of water so that the manometer column rises 60 cm. per minute. Read the manometer when the starch jelly suddenly breaks. Tests are made in triplicate and averaged. The gel strength is reported as the height (in centimeters) reached by the liquid in the manometer at the breaking point of the gel, for a given concentration of starch to water in the paste.

c. *Saare Disc Method.* Among the first tests proposed for the evaluation of a cold paste was that of Saare and Martens (24), in which the weight required to withdraw a disc from a gel in which it was imbedded was determined. A modification of the method has been used by the author for several years. Details of the modified method follow: Starch is cooked in the same manner as described for the Scott test, a higher ratio of starch to water than in the latter test being used. For unmodified corn starches, 22.4 g. are used per 280 cc. of water. For modified starches between 30 and 50 g. of starch may be used, depending on the extent of modification. Immediately after the 15 minute cooking period, the starch is poured into a glass vessel, and the latter is filled to a definite mark. A circular metal disc of known diameter is suspended in the paste by means of a metal rod connected to the center of the upper surface of the disc. The metal rod is crooked at the top and is hung over a bar which rests on the top edges of the vessel holding the pastes. The length of the rod is such that, when suspended over the horizontal bar, it permits immersion in the paste of the test disc to a depth of 3 cm. A thin film of light oil is placed on top of the paste as soon as the disc has been inserted and properly centered. The test vessel and contents are now placed in a constant temperature bath at 25°C. and held in the bath for 24 hours. At the end of this time the test vessel is removed and placed on a bridge over one pan of a large, but sensitive, beam balance. A specially made hook suspended from the beam of the balance just engages the crook in the rod attached to the disc. The temporary bar support for the suspended disc is of course removed first. Small size shot are added at a fixed rate to the other pan. The weight of shot is noted at the time when the disc fractures the gel. Figure 50 shows an assembly for the determination of gel strength.

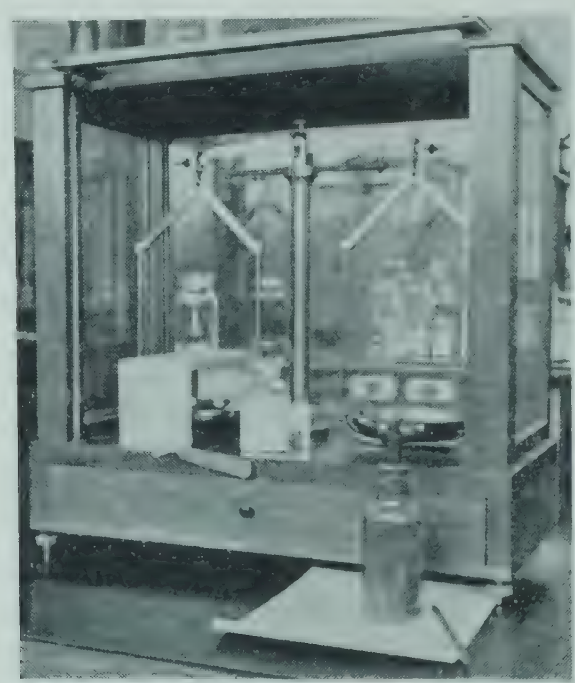


FIG. 50. Apparatus for starch gel strength by modified Saare method
Photograph by H. Meisel.

Some types of starch gels show a considerable deformation before a fracture actually occurs, as intimated above. One should not be misled into mistaking such deformations for an end-point in the test under discussion. An actual visible fracture is taken as the end-point.

The weight of shot measured, less the weight of disc and all connections to the beam of the balance, divided by the exact area of the lower surface of the disc (about 3 sq. cm.) is taken as the gel strength. The result is expressed in grams per square centimeter for a given concentration of starch.

One shortcoming in equipment so elementary in design is that no guides are provided to maintain the rod attached to the surface of the disc in a perfectly vertical position. Some skill and practice is therefore required in adjusting this rod to a vertical position and maintaining it so during the period of test. Otherwise some slippage of the disc may result as the disc is pulled from the gel.

The method is, none the less, adapted to routine testing in practice and has a precision of 2 to 3%. This precision is more than ample for industrial practice, as can be appreciated from the fact that industrial grades of thin boiling starches vary about 20% in gel strength when one fluidity, *e.g.* a tarch of 20 fluidity, is compared with the next, for instance a starch of 40 luidity. The accuracy of this method has been found greater by the writer han the Tarr-Baker or any other method of evaluating limits of strength in a gel. With such simple equipment many tests may be set up simultaneously by securing identically made discs and calibrated vessels to hold the pastes or testing. As for most other tests for paste evaluation, the major part of he experimental error involved is in the preparation of the paste, or gel, o be tested.

d. Penetrometers. The use of various penetrometers has been proposed, but most of these are not sufficiently sensitive to distinguish even between the various grades of acid-modified industrial starches. The writer's experience with the Bloom gelometer used extensively in the control of glue and gelatin manufacturing gives approximately the same order of values for starches with fluidities of 20, 40, and 60, all conditions of test being equal. Alexander (25) gives a discussion of this instrument and its use.

One type of penetrometer is, however, as sensitive and as accurate as the modified Saare method of determining gel strength. The paste is cooked as in the Scott test, between 20 and 35 g. per 280 cc. of water being used, depending on the modification of the starch. After being cooked, it is immediately placed in a closed, wide-mouth container for storage in a constant temperature bath. At the end of the aging period the cover of the vessel is removed and about a ¼ inch layer of the gel is cut off and discarded. The plunger of the instrument is now adjusted so that it just rests on the top surface of the prepared gel. A weight is imposed on the plunger in a receptacle on top of a rod, which is held in a true, vertical position by several guides. The bottom of the rod is attached to the top of the plunger. The latter consists of a sharpened, hollow metal tube that is highly polished. This tube is similar in appearance to a large cork borer. The plunger is released, and the time, in seconds, required for the hollow tube plunger to cut into the gel up to a certain depth is noted. This depth is determined by three markings on the rod or shaft affixed to the plunger. One of the guides is movable and is used as a point of reference. It is set at the lowest mark on the shaft just before the plunger is released. The time is noted when the middle mark passes and when the upper mark on the shaft reaches the point of reference. An instrument of this type has been constructed and used by the authors' associate, F. W. Fuchs (Fig. 51).

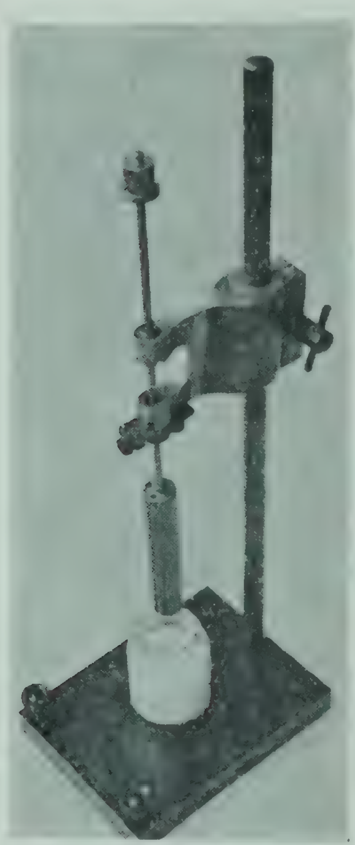


FIG. 51. Fuchs gel tester for starch pastes. (Courtesy of F. Fuchs; photograph by H. Meisel.)

e. Rigidometers. A very satisfactory instrument for determining the elasticity or the rigidity of a gel has been described by Brimhall and Hixon (26). The method of employing the rigidometer with starch pastes has been fully described by these authors.

The essentials of the instrument are as follows: A glass tube is suspended by means of a torsion wire into a column of paste, and the latter is allowed to gel and age at a fixed temperature. The torsion wire is suspended from a circular scale, quite similar to the arrangement in the MacMichael viscosimeter. At the end of the aging period the cylinder holding the column of paste is placed in the stand of the instrument which holds it in a fixed position. By means of a dial knob attached to the face of the circular dial, a small twist is developed in the wire. The angle of twist is noted. The corresponding angle through which the tube which is embedded in the starch gel moves is determined by the angular deflection of a beam of light which falls on a mirror attached to the glass tube. Determinations are repeated for different degrees of twist of the torsion wire. The originators of the method have given the method for determining the rigidity from the values observed. The angular degrees of twist, δ, applied to the wire are plotted against the corresponding deflections, ω, in angular degrees. The slope of this curve δ/ω is substituted in the following equation,

$$E = \frac{N}{4\pi h}\left(\frac{1}{R_0^2} - \frac{1}{R_1^2}\right)\frac{\delta}{\omega}$$

where E is the modulus of rigidity in dynes per square centimeter, N the torsional moment of the wire used, h the height of the paste on the inner cylinder, and R_0 and R_1 the radii of the inner tube and outer cylinder respectively. The wire used is standardized by measuring the oscillation time of a suspended disc of known mass. Then $N = 4\pi^2 MR^2/2T^2$ where T is the period of oscillation, M is the mass of the disc, and R is its radius.

In applying a torque to the wire, one should not turn through an angle so great that the structure of the gel will be in any way permanently injured.

6. **Plasticity in Pastes.** Several attempts have been made to measure the plasticity of starch pastes. In general, these have been based on the concepts outlined by Bingham (27). The rate of shear is plotted against shearing stress, and the curve is extrapolated to zero rate of shear in order to obtain the "yield shear value." Hypothetically, this value is the force applied in overcoming plasticity in a fluid before the paste begins to flow. Application of the method to starch, however, presents certain difficulties. For example, Porst and Moskowitz (28) find that the curve obtained by plotting the rate of flow of a starch paste in a Bingham-Greene type plastometer against the applied pressure is not a perfectly straight line. It is difficult, therefore, to extrapolate these curves to zero rate of flow. Furthermore, it has been found by Farrow *et al.* (29) and by Hatschek (30) that flow actually takes place below the yield value estimated, and thus the latter does not agree with Bingham's definition and has no physical meaning. It has also been observed that the results obtained with a starch paste depend to a large extent on the size of the capillary through which the starch is forced to flow.

The results plotted on a logarithmic scale give a more nearly linear line which can be extrapolated with greater ease. Farrow *et al.* (29) therefore propose (and Rabinowitsch (31) gives a mathematical justification for the procedure) to plot the logarithm of the stress applied to the paste ($RPg/2L$) against the logarithm of the rate of shear produced ($4v/\pi R^3T$). If the slope of this line is denoted as M, and C is the point of interception, then the mathematical equation becomes

$$\log C = \log 4v/\pi R^3T - M \log RPg/2L$$

Not only do the results, so expressed, give a fairly straight line, but the position of the line is independent of capillary size. Moreover, it would appear that with suitable methods other types of viscosimeters may be used and the results, calculated in terms of shear and stress, may be correlated and expressed by the same constants as determined with capillary viscosimeters.

Brimhall and Hixon (32) have applied the above equation to a study of the flow characteristics of pastes made from a variety of starches and have shown that the equation holds for the results obtained by use of a specially designed viscosimeter of the capillary type. Their method and equipment would seem to be ideally suited for the measurement of flow in starch pastes

Pastes at 5% concentration are cooked in a Pyrex test-tube suspended in a hot water heating bath. The test-tube is provided with a stopper through which is inserted a stirrer or, at the end of the cooking period, a capillary pipette. A 40 minute heating period at 90°C. is recommended. The paste is then cooled to 25° C., and the capillary pipette, with a capillary of known radius in centimeters (R) and known length (L) is introduced. The pipette is connected to a three-way stopcock, so that suction may be applied to fill the pipette to a given volume (V). The time of flow in seconds (T) is measured for various applied pressures, obtained from an air pressure line with a relief-regulating valve, which are read on a water manometer as centimeter head of water (P); g is the acceleration of gravity.

Capillaries should be between 0.035 and 0.075 cm. in radius, so that the time of flow is kept in the range between 30 and 200 seconds. For unmodified or very viscous starches, the weight of starch used should be adjusted so that comparable rates of flow are obtained. The cooking and cooling procedure should be standardized and the technique adopted rigidly followed.

More recently, Green (33) has reported that although many systems give a hyperbolic curve when rate of flow (V/t) is plotted against pressure applied in a capillary viscosimeter, which result is due to a very gradual change from plug to laminar flow as the pressure is increased, some systems, the "Bingham bodies," give fairly straight lines when the angular velocity of a rotational viscosimeter (above a certain low level) is plotted against the torque applied to the system. This has been the writer's experience (34) in the study of aqueous mixtures of clay, deflocculating agents and starch pastes, using a MacMichael viscosimeter, as mentioned above. However, in a review of the rheology of simple aqueous starch pastes made from a variety of starch products, Fischer and Lindsley (35) could find no evidence for linearity in the curves for torque plotted against rate of shear, nor any indication of an interception on the torque axis corresponding to a yield value when a rotational viscosimeter, such as the Corn Industries instrument, was used for measurement. Moreover, these workers question whether the positive curvature shown by these graphs are an indication of true thixotropy, or whether the result is due to relatively non-reversible changes in the colloidal structure of the pastes.

Goldberg and Sandvik (36) have described a novel instrument for measuring the rheological properties of elastic fluids. The paste is placed in the annular space formed by two concentric cylinders, one of which is oscillated mechanically at frequencies ranging from 10 to 6000 cycles per minute. After the resonance frequency and maximum amplitude of the system have been observed the elastic constants of the system may be calculated. A modified apparatus allows measurements to be taken while the sample is being sheared at various rates and rapid changes of shear modulus may be recorded photographically.

7. **Paste Shortness.** This characteristic is one of the most commonly discussed properties of paste in industrial applications; yet the property is rarely evaluated except by casual inspection. As the product is being cooked or otherwise prepared, a paddle, ladle, or rod may be immersed in the paste and withdrawn to note whether the flow therefrom is stringy or short (which is the opposite property) in character.

If the restricted flow of such pastes is observed by the aid of a stroboscope, as for example the dropwise delivery from a small orifice, the shape of such drops as they emerge will be seen to vary progressively as pastes with progressively greater shortness are tested. Native corn starch pastes are comparatively short, whereas tapioca or potato starch pastes are normally stringy. Proper mixtures of the two types may be made to obtain pastes with intermediate properties.

The above observation may be made the basis of a test for shortness. The author has used and prefers, however, a more simply executed test, a description of which follows: Depending on whether shortness is to be estimated on the hot paste or cold paste, a weight of starch is mixed with that amount of water which, after the cooking in procedures otherwise comparable to a particular industrial application and at the temperature selected, will deliver 50 cc. from the orifice of a Scott viscosity cup filled with 200 cc. of the paste in 100 ± 1 second. When these preliminary conditions have been fulfilled, the test is repeated, with the lowermost point of the Scott cup orifice about 2 cm. above the graduated cylinder used to receive the paste. The orifice is opened, and the eye is trained to the space between the orifice and cylinder. At first, the delivery of the paste will be observed to be a continuous thread. After a period, the delivery appears to be discontinuous, in part. Eventually, a point is reached at which the outflow changes quite definitely to a dropwise delivery. At that instant, the number of cubic centimeters of paste collected in the graduate is noted. This value may be taken as a relative measure of shortness; the lower the value, the shorter is the paste. Certain starches, modified to increase the short property of their pastes, will give values of 15 to 20. Native corn starch exhibits a hot paste shortness of 50. Unmodified tapioca will give a value of 125 under the same conditions of testing. The method was developed empirically after comparative observations on the hot paste viscosity of various starches by the Scott test.

8. Syneresis or Water Retention in Starch Pastes. Many pastes after aging will show a tendency to "bleed" or separate a liquid phase. This is particularly true of short gels with comparatively low concentrations of dry starch solids. This property is rather difficult to evaluate, especially in cases in which sufficient starch has been added so that a firm gel results. An evaluation may be obtained directly in some cases and indirectly in others by preparing a paste at such a concentration that it will remain fluid when cooled and aged. Two methods are suggested, each of which depends on the speed of filtration of the liquid phase.

In the first, a suspension of a selected clay is made up by mixing 30 g. of clay with 75 cc. of water. After a thorough mixing of the clay, sufficient starch paste is added so that the ratio of starch solids to clay is 15 : 100. Hence, 4.5 g. of starch are added. The total water present is now adjusted to 175 cc., and the mixture is stirred in a covered, high speed mixer for 10 minutes, whereupon it is transferred to a funnel fitted with a standard filter paper. The number of cubic centimeters collected in a given time interval is taken as a measure of the relative water retention of the starch. Obviously, the clay and filter paper used should be standardized as far as possible. A good grade of English clay should be selected, and a filter paper such as Munktell's No. 3 should be used.

For the second type of test, a stock supply of light weight, bleached but unsized paper stock should be procured. This is cut into squares measuring about 3 cm. on each edge. Just before the test is to be performed, one face of the paper (the wire face) is dusted with an indicator which changes color in the presence of moisture. For example, a powdered, intimate mixture consisting of 45 parts of cane sugar, 5 parts of soluble starch, and 1 part of du Pont N. E. methyl violet may be used as an indicator. The paste to be tested is thoroughly stirred up, and about 25 cc. are placed in a small porcelain dish. The prepared paper is placed on the surface of the paste with the dusted side up. With the aid of a reading glass, the time in seconds is noted for the indicator to change color. This value may be taken as a relative index of the water retention of the starch when a paste has been formed.

The starch is gelatinized in the manner recommended for preparing a paste for the Scott test. Ten grams of starch is used per 280 cc. of water. After the mixture is cooked, the covered beaker containing the paste is transferred to a bath at 25° C. At the end of 3 hours the paste is removed for test. About ten observations on a paste should be made and the results averaged. The results are expressed as seconds of water retention for a given concentration of starch. Water retention tests may and should also be determined at different starch concentrations. A more complete picture of the measured characteristic is given by the slope of the curve obtained by plotting water retention against starch concentration in the paste. Variables which require standardization are the paper and indicator used and a definition of the end-point with the indicator.

Water retention of pregelatinized starches, such as the roll-dried corn starch products of commerce, are very readily determined as follows: 5 g. of product, measured on a dry solids basis, are wet with a small amount of distilled water to form a dough. More water is added with stirring to form a suspension which is quantitatively transferred to a 100 cc. graduated cylinder. Sufficient water is now added to make the total volume up to the 100 cc. mark. After a complete mixing of the contents, the cylinder is allowed to stand at rest for 24 hours at 25°C. At the end of this time the line of demarcation is noted where the starch product appeared to have settled out from the water phase. The apparent number of cubic centimeters of the insoluble phase divided by 5 is taken as an index of the water retention of the product, per gram.

9. Gelatinization Temperature of Starch. Many methods have been proposed for the measurement of this important paste characteristic of a starch. Possibly the reason that divergent values are found in the literature for a given starch is the poor definition of the term. Naturally the choice of method for making the determination will, in a measure, depend on one's conception of the gelatinization point. For example, in gelatinization the granules first swell and finally collapse or tend to disintegrate if the heating is extended. If a technique is employed which takes advantage of the fact that the granules swell, such as by observing increases in the viscosity of heated suspensions of starch, it will be observed that the viscosity increases over a broad range of temperature. It is a matter of judgment, therefore, to locate the central portion of the range or the portion where the viscosity is increasing at a maximum rate. Simultaneous examination of these pastes by means of a microscope, moreover, reveals several additional complications. Swelling of granules begins before the effect of increased viscosity becomes apparent by the use of many viscosimeters employed in practice. Moreover, in some cases, the viscosity continues to increase apparently after the granules have reached their point of maximum distention. Finally, not all the granules in many types of starch swell in exactly the same temperature range. In general the larger, more fragile granules swell at a lower temperature than the smaller granules of the same type of starch. In varieties in which there is a wide range of granule size and the sample is composed chiefly of the smallest and largest members, with few granules of intermediate size, a double gelatinization point may be observed by several methods.

Another characteristic of swelling granules is that they lose their activity in polarized light. The crosses commonly observed in native starch granules gradually become less distinct as a water suspension of the starch is heated. Finally they disappear altogether. Indefinite end-points are, therefore, the usual result of the use of methods based on this property, for much the same reasons as given above (37). What is more confusing, however, is that loss of birefringence cannot always be correlated with the change in viscosity. Many corn starch samples will have lost birefringence in all granules, 10° or more under the temperature at which significant increases in viscosity start to become apparent in some continuous viscosimeters (38).

Another characteristic of starch suspensions in water, heated to their gelatinization point is that the suspension becomes less opaque. This characteristic has also been made the basis for methods to determine the gelatinization temperature of starch. Some starches, however, give much more translucent solutions as they gelatinize than others. This consideration must be kept in mind in outlining methods for comparing various starches by their light transmission at various degrees of gelatinization.

The hydrogen ion concentration is a variable which should be controlled in all methods, since the temperature at which any starch gelatinizes is a function of the pH. Gelatinization temperatures reported in the older literature, especially for corn starch, are practically worthless inasmuch as the pH was not controlled. Most corn starch, several decades ago, was made by an alkaline process and, as a rule, was finished at hydrogen ion concentrations higher than those now considered normal for corn starch. The presence of other specific ions or molecules should also be considered. The degree of gelatinization, other conditions being constant, is affected by the rate of stirring the sample, to a lesser extent by the rate of temperature increase, and, in extreme cases, by the ratio of water to starch present in the test samples. Samec (39) has reviewed some of the earlier methods used to determine the gelatinization temperature and has discussed at length the control of factors which alter the gelatinization temperature of a starch.

More recently, Morgan (40) has elaborated on a method based on the change in light transmission of starch slurries during the period of paste formation by heat. A photoelectric method is used to measure the change from cloudiness to translucency which occurs when a great variety of starches is heated with water. These include native white potato, wheat, tapioca, rice, corn, sweet potato, sago, and Florida arrowroot starches.

About 0.33 g. of a starch sample is rubbed up in 65 cc. of water and poured into a large test-tube. A small stirrer is set in the slurry and turned on. The tube and contents are immersed in a glycerol bath which gradually raises the temperature of the starch 2.5° C. per minute. Light from a 100 watt projection bulb passes through a collimating lens, on through the tube in which the paste is formed, and onto the photoelectric cell. The intensity of the light source is maintained by a constant voltage regulator. The illumination is initially adjusted, just before a determination, by use of a slide-wire resistor, to a standard value of 250 microamperes or 500 foot candles. A microammeter is attached to the photocell. The values for light transmitted, in microamperes, are plotted against the temperature (°C.). Characteristic curves are obtained from each starch. Modified starches such as the so called oxidized starches and acid-modified starches of commerce show progressive alterations in the curve.

Wheat starch shows a double gelatinization range referred to above. Sago and Florida arrowroot show an initial increase in opacity which is ascribed to a disintegration of aggregates into individual grains. It is claimed that the start of paste formation indicated in this method by an abrupt rise in the transmission corresponds to the first appearances of paste formation as shown by a decrease in the number of granules showing birefringence. This would seem to be confirmed by comparing the data of Morgan on corn starch with that given by Schoch (38). Although Morgan directs attention to the temperature at which paste formation begins, the curves can very readily be used for an estimation of the half wave height, which might be taken as the gelatinization point by this method. Wheat and starches behaving similarly would have two gelatinization points.

However, when these curves are compared with those obtained by plotting viscosities against temperature, *e.g.* replotting Brabender amylographs on rectangular coordinate paper, and estimating the half wave height of these curves, a discrepancy in the result obtained by the two methods is disclosed.

For example, by the Morgan method the gelatinization point of corn starch appears to be 77° C., whereas by the Brabender method a value of 85° C. is obtained. Hence gelatinization point is mainly a matter of definition. From an analysis of the supplementary data available (38, 40) it would appear that the first method is the more reliable. Using the Brabender Amylograph for the purpose described, one should take as the gelatinization point that temperature at which the graph first shows a decided change in slope upwards. For corn starch, unmodified, at normal pH this would be 78° C.

To show the extreme variation in the calculated results obtained with the different methods employed to determine the gelatinization point of starches, reference is made to the recent work of Mullen and Pacsu (41). In presenting a review of the general subject of gelatinization, these authors used an instrument of the type described by Caesar (12) to measure the increase in the consistency of 15% starch pastes, and they employed a temperature increase of 3° C. per minute. When the increase in the wattage input to the stirring motor is plotted against the temperature, very sharply rising curves are obtained in the region of gelatinization. The half wave temperatures found are as much below those obtained from results on light transmission as the half wave temperatures of the Brabender amylographs are above. For example, for corn starch the gelatinization temperature (defined by the authors as the temperature at which the rate of increase in consistency reaches its greatest value) is given at 68.5°C. in pure water. The results for other starches are comparatively low. Furthermore, the authors note that for starches studied, potato, tapioca, wheat, corn and rice, the gelatinization temperature in water is inversely related to the average granule size of the sample. According to this method it would seem that the probable gelatinization point is more nearly the temperature at which the starch exhibits its maximum consistency.

Although the technique of Morgan is recommended for more careful studies, the author has obtained comparable results with a very simple and quickly performed procedure used for many years. In routine work the experimental error is about 1° C. for an experienced operator.

Starch slurries are made up at 10% concentration for unmodified corn starch and at concentrations for other starches depending on their relative hot paste viscosity as compared to that for corn starch. For example, for the average, acid-modified thin boiling corn starch, 15% concentration is recommended. Unless results are reported to the contrary, the pH of the slurry is adjusted so that the final starch has a pH of 5.0 to 5.5. The slurry is placed in a metal cup provided with a stirrer and thermometer. The cup is then placed in a water bath having a temperature about 5° C. under the anticipated gelatinization point. The stirrer and heating unit are turned on gradually. When the temperature of the slurry is about 10° below the anticipated gelatinization point, the rise in temperature is regulated at 1°C. per minute. Mechanical stirring is discontinued and the stirring continued with a glass rod, by hand. The temperature at which the first thickening effect of the slurry is discerned and also at which the paste has become so thick that further increases cannot be noted by inspection is recorded. For most starches this range is about 4-6°. The average of the two temperatures is taken as the gelatinization point. For unmodified corn starch at pH 5.5 this value is 77° C.

Beckord and Sandstedt (42) have developed a simplified method based on light transmittance, by use of the spectrophotometer. In this procedure, gelatinization temperature of starch samples was found to be unaffected by rate of heating over the comparatively wide range of 0.15 to 2.5° C. per minute. It is of interest to note that large and small wheat starch granules gave curves essentially similar in character, indicating that the two types of wheat starch granules have similar gelatinization characteristics.

10. Protective Colloid Effect of Starch Sols. Specific tests of this nature are difficult to outline, inasmuch as the effectiveness of the starch will depend on the properties of the material to be suspended, the nature of the suspensoid to be precipitated, whichever the case may be, or on the properties of the nonmiscible liquid to be emulsified. Also, it is possible that additional substances which may be present may affect the suspending properties of the starch product. It would seem more sensible to standardize, as tests, small scale operations comparable to the exact use to which the product is to be put.

Starches are used as suspending agents, and one of the chief uses of this type is to suspend clays in preparing heavy sizes of coatings. An illustrative test given below is based on this use. Starch products are used as flotation agents in certain mining operations and as precipitants in others. In the latter instance, the sedimentation of solids from the wash water from coal may be noted. Starches are used as emulsifying agents, particularly in such food products as salad dressings. Starches are claimed to be foaming agents, particularly by those who, because of poor control in the preparation of adhesives or sizes from starch, are perpetually troubled with foaming products. In such cases investigated by the writer, however, conditions of foam could always be traced to some extraneous material such as might be present in the water supply at the point of use, unanticipated high pH indicative of free alkali, or even accidental impurities in the starch product or to reagents or additives which should not have been used. A high content of soluble proteins in enzymes of weak diastatic power is an example of the latter. Moreover, in instances in which the author has deliberately attempted to make a marketable foaming agent from starch, these experiments have met with utter failure. In general, it may be said that starch is not a foaming agent and is of such weak emulsifying power that rather large concentrations of starch sol are required to hold up such fats as the vegetable oils (43, 44). Oxidized starches are among the best types of starch for protecting suspensoids; unmodified corn starch, particularly if it is partly gelatinized and then dried in a manufacturing process, is a good sedimentation agent for many suspensoids.

The clay-suspending ability of a starch may be conveniently determined as follows: 1 g. of the starch product, dry weight basis, is thoroughly gelatinized in about 75 cc. of water by a standard cooking procedure and cooled. An excess of English clay is now stirred into the starch, for example 2 to 3 g. After thorough mixing, the mixture is quantitatively transferred to a standard 100 cc. graduate with sufficient water to make a total volume of 100 cc. After a second thorough mixing of the contents, the graduate is stoppered and allowed to stand 20 hours at 25° C. The topmost 80 cc. are now withdrawn, and a determination of the dry solids is made by evaporation on sand and then by heating for 4 hours at 120° C. in a vacuum oven. The weight obtained divided by 0.8 and minus 1.0 is taken for comparative purposes as the relative clay-suspending ability of the starch at the pH noted for the suspension.

Alkalies in general, such as sodium carbonate, borax, and tetrasodium pyrophosphate, are fluxing agents for clay. Obviously, therefore, the pH is a variable in the above determination which should be controlled. The clay used for testing should be standardized, as some clays are more difficult to suspend than others.

11. Supplementary Tests on Paste. Quite frequently supplementary tests are performed which supply additional information of practical importance. Many of these are extensions or combinations of the types of tests already given. For example, it frequently becomes important to know the comparative rate of gel formation in a starch paste at a given temperature. Otherwise, on the basis of a preliminary examination, a starch might be incorrectly applied. In such cases, a series of duplicate tests for gel strength may be set up, the same starch being used in each case. At stated time intervals a sample is removed from the constant temperature bath and an observation is made. Gel strengths are then plotted against the time measured after the cooking and cooling operations. A curious effect may be observed with the procedure outlined. The presence of very small amounts of fats increases

the rate of gel formation in corn starch. After a short period of time, such a starch will show a higher gel strength (and cold paste body) than a defatted corn starch. However, after longer periods of time the defatted starch will surpass the other starch in gel strength (and cold paste body).

One of the most important characteristics of a starch for many industrial uses is its change in properties under the influence of mechanical stresses such as pressure, shearing action, and so forth. In many mills employing starch products it would seem that very little thought had been devoted to the location of equipment for the preparation of the starch. Accordingly, paste kettles will be found in out of the way places, frequently in the basement or in other parts of the building unsuited for any other use. Equipment for application of the prepared adhesive or size may therefore be located at some distance from such paste kettles. Frequently, rather considerable pressures are required to pump these pastes to their point of use. It is important to know to what extent the viscosity of such a paste may be expected to be reduced after such treatment, whether its tendency to gel will be increased when the pump and pipe lines are left full of paste during a periodic shutdown of the plant as over week ends, and so forth.

Again, it may be observed that some portion of a moving part of the mechanism which applies a paste is of such poor design that undue shearing forces or cutting actions are set up in the starch supply. These tend to create a nonuniformity in the viscosity of the starch paste. For example, supply boxes at the machines are often provided with an overflow to large supply tanks. In such cases, the first part of a batch of adhesive or size may be of substantially higher paste body than the last of the batch.

No standard tests are suggested. It is recommended that equipment be set up to duplicate the particular physical stress observed which is to be compensated for by selection of a specially made starch product. Viscosity, or cold paste body, and other pertinent properties are observed before and after stated periods of treatment. Care should be taken that the pH and temperature correspond with those of the paste used in the practical application.

12. **Color of Starch.** The color of starch is conveniently measured by the use of the Brice photometer or a similar instrument. The percentage of light reflected from the smooth surface of the powdered starch sample is determined by means of a photoelectric cell. When the instrument is balanced for a standard white surface, usually one prepared from MgO, which is assumed to give 100% reflectance, the percentage reflectance of the starch may be read directly. It is preferable to make two determinations, one with a green filter in the path of the light source and the other with a red filter.

Both filters are supplied with the instrument. The percentage deviation from white may then be expressed as

$$100 - \frac{\%\ \text{reflectance by green light}}{\%\ \text{reflectance by red light}} \times 100$$

In the determination of color by this method, errors introduced, for example, by the brilliance of the starch sample, are minimized.

The visual inspection methods depend on the use of standard samples. Other starches are then graded by direct comparison with these standards. There are three general methods which require very little practice to enable one to match an unknown with the standards. The first two require that the starch be dry and powdered, preferably to a given range of particle size, as for example that passing through 200 mesh copper sieves and retained on 300 mesh.

In the first, a small amount of sample (about 0.5 g.) is placed in the center of a jet-black sheet of plate glass. Small samples of standards are now placed in a circular arrangement around the unknown and at such distances that when a sheet of clear plate glass is gently pressed on all starch samples the surface of the starches will be continuous, one with the next. This surface is examined by reflected daylight, free from direct sun rays or reflected glare. The unknown is said to match that standard in which no line of demarcation can be discerned between the surface of the unknown and the standard.

The second method is the so called "stab test." The standard samples are placed in uniformly shaped glass bottles of equivalent size. The unknown is placed in a similar bottle. Each bottle should contain the same volume of starch. They are placed on a dark colored stand in reflected sunlight, and a hole is formed in each sample of starch, in turn, with a glass rod which is forced gently down from the top of the bottle until it strikes the bottom. Care is exercised in removing the rod, lest the hole formed become materially larger or smaller through a disturbance of the surrounding starch. The eye is now trained on the various holes made, and the color perceived in the unknown is matched against the standard which most nearly approaches it.

The third method involves cooking the starches in dilute suspension, *e.g.* 2 g. in 100 cc. of water, in glass equipment and by a standard procedure. When cooked, the standards and unknown are cooled to 25° C. and compared by transmitted and reflected light after a given interval of time. Observations by this method may be complicated by opacity which in turn depends on the type of starch, degree of gelatinization, and retrogradation effects. The color developed may also be influenced by a variation in the pH of the sample. Moreover, color comparisons by this method may not be correlated with those obtained on the dry samples. However, the former are considered more reliable when the starch is to be applied as a paste rather than used in dry, powdered form.

More recently a test has been proposed in an attempt to evaluate the color of a starch, in particular of corn starch, quantitatively on an independent scale. The method presupposes that all of the color of starch is imparted by carotenoids such as those present in unmodified corn starch. This, of course, is not necessarily so, and the test must therefore be used with certain reservations in mind.

Ten grams of starch, dry basis, are shaken in a glass-stoppered flask with 100 cc. of 70% alcohol by volume. The flask is then centrifuged and the color of the clear liquor determined in the Lovibond type colorimeter on the Lovibond scale, with 70% alcohol as the standard reference fluid.

13. Odor of Starch. The odor of corn starch products is noted by inspection. pH and moisture determinations are first made, since these are variables affecting the development of odor. A clean, 8 oz. glass-stoppered bottle is half filled with the sample which is then placed in a constant temperature oven at 60° C. It is gently shaken and inspected once a day, and the length of time in days during which the product remains stable at a given moisture and pH is recorded.

14. Mobility of Dry Starch. A test adapted to the equipment found in most laboratories is as follows: 200 g. of the powdered starch are placed in a 200 mesh copper sieve such as the standard type supplied by the W. S. Tyler Company. The circular sieve is supplied with a cover and a receptacle to catch the starch passing the seive. The assembly is placed in a Rotap shaker and shaken for exactly 30 seconds. The percentage weight of starch passing the sieve is taken as an index of its mobility. Starches considered as mobile will pass 85% or more of the 200 g. placed on the sieve. The pH and moisture content of the starch should be noted, since they may be variables affecting mobility.

Occasionally the test is carried out on a larger scale in the mill. For example, 100 lbs. of starch may be dumped on a standard sieve consisting of a 48 mesh wire gauze attached to an eccentric making 120 r.p.m. If the sieve is of such size that about a 6 in. layer or so is present on the screen when the bag of starch is dumped, an immobile starch may require as much as 30 minutes for the starch to pass the sieve. A mobile starch will pass through the sieve in 2 to 5 minutes.

15. Miscellaneous Tests. Other miscellaneous tests are applied in industry for the evaluation of a starch for general or for a specific use. Many of these are described in the sections on reactions or uses. For example, a control is maintained for starches specially prepared for diastatic liquefaction by the user, and it is the common practice to evaluate all batches of starch produced by some standard method for their susceptibility to such liquefaction.

Naturally, industrial starches are analyzed for ash, protein, fat, pH, and other characteristics, and a close control is kept by microbiological tests. The usual chemical tests are based on the Methods of the Association of Agricultural Chemists which are revised and brought up to date periodically. Many analytical methods based on the chemical properties of starch products are given in Chapter XXIV. Bacterial counts, such as for the various classes of thermophiles, are usually made in accordance with procedures approved by the food manufacturer or a group of them, as, for instance, the National Canners' Association.

Occasionally specifications allow only an infinitesimal minimum of some substance; *e.g.*, iron. In most of these cases, there is little difficulty in finding a suitable method in some standard text on analytical chemistry.

Sair and Fetzer (45) have made a careful review of methods for the determination of moisture in starch. They find that either a toluene distillation or drying in a vacuum oven at 100° C. gives reliable results as to the true moisture content.

BIBLIOGRAPHY

1. MacNider, G. M., *Ind. Eng. Chem.*, **4**, 417 (1912).
2. Kerr, R. W., U. S Patent 2,108,862 (1938).
3. Ermen, W. F. A., *J. Soc. Chem. Ind*, **26**, 501 (1907)
4. Chrzaszcz, T., and Janicki, J., *Biochem. Z.*, **256**, 252 (1932).
5. Alsberg, C. L., and Rask, O. S., *Food Research Inst.*, Stanford University (Jan., 1924).
6. Kerr, R. W., U. S. Patent 2,268,215 (1941).
7. MacMichael, R. F., *Ind. Eng. Chem.*, **7**, 961 (1915).
8. Gallay, W., and Bell, A. C., *Can. J. Research*, **B14**, 381 (1936)
9. Hoeppler, F., *Chem.-Ztg.*, **57**, 62 (1933).
10. Wobser, R., and Müller, F., *Kolloid-Beihefte*, **52**, 165 (1941).
11. Valenta, E., *Chem.-Ztg.*, **30**, 583 (1906).
12. Caesar, G. V., *Ind. Eng. Chem.*, **24**, 1432 (1932).
13. Radley, J. A., Starch and Its Derivatives, New York (1940).
14. Müller, G. F., *Cong. intern. tech. et chim. inds. agr., Compt. rend. 5e Congr. Budapest*, **2**, 539 (1939).
15. Anker, C. A., and Geddes, W. F. *Cereal Chem.*, **21**, 335 (1944).
16. Barham, H. N., Wagoner, J. A., and Reed, G. N., *Ind. Eng. Chem.*, **34**, 1490 (1942)
17. Kesler, C. C., and Bechtel, W. G., *Anal. Chem.*, **19**, 16 (1947).
18. Bechtel, W. G., *Cereal Chem.*, **24**, 200 (1947).
19. Bechtel, W. G., and Kesler, C. C., *Paper Trade J.*, **125**, 35 (1947)

20. Higginbotham, R. S., *Shirley Inst. Memoirs,* **20**, 1 (1946); *J. Textile Inst.,* **38**, T131 (1947); *Textile Tech. Digest,* **3**, 205 (1946).
21. Buel, H., *Intern. Congr. Applied Chem., 8th Congr. Washington and New York, Original Communications,* 13, 63 (1912).
22. Baker, G., *Ind. Eng. Chem.,* **18**, 89 (1926).
23. Tarr, L. W., *Univ. Delaware Agr. Exp. Sta. Bull.,* **142** (1926).
24. Saare, O., and Martens, P., *Z. Spiritusind.,* 26, 436 (1903).
25. Alexander. J., Glue and Gelatin, New York (1923).
26. Brimhall, B., and Hixon, R. M., *Ind. Eng. Chem., Anal. Ed.,* **11**, 358 (1939).
27. Bingham, E. C., Fluidity and Plasticity, New York (1922).
28. Porst, E. G., and Moskowitz, M., *Ind. Eng. Chem.,* **14**, 49 (1922).
29. Farrow, F. D., Lowe, G. M., and Neal, S. M., *J. Textile Inst.,* **19**, 18 (1928).
30. Hatschek, E., The Viscosity of Liquids, London (1928).
31. Rabinowitsch, B., *Z. physiol. Chem.,* **145A**, 1 (1929).
32. Brimhall, B., and Hixon, R. M., *Cereal Chem.,* **19**, 425 (1942).
33. Green, H. G., *J. Colloid Sci.,* **3**, 129 (1948).
34. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, 360 (1944).
35. Fischer, E. K., and Lindsley, C. H., *J. Colloid Sci.,* **3**, 111 (1948).
36. Goldberg, H., and Sandvik, O., *Anal. Chem.,* **19**, 123 (1947).
37. Alsberg, C. L., and Rask, O. S., *Cereal Chem.,* **1**, 107 (1924).
38. Schoch, T. J., *Cereal Chem.,* **18**, 121 (1941).
39. Samec, M., Colloid Chemistry of the Starches, Dresden, 168 (1927).
40. Morgan, W. L., *Ind. Eng. Chem., Anal. Ed.,* **12**, 313 (1940).
41. Mullen, J. W., II, and Pacsu, E., *Ind. Eng. Chem.,* **34**, 807 (1942).
42. Beckord, O. C., and Sandstedt, R. M., *Cereal Chem.,* **24**, 250 (1947).
43. Goikhman, N. G., *Khim. Referat. Zhur.,* No. 5, 109 (1940).
44. Goikhman, N. G., *Farmatisiya,* **10**, 10 (1939).
45. Sair, L., and Fetzer, W. R., *Ind. Eng. Chem., Anal. Ed.,* **14**, 843 (1942).

SECTION III. PROPERTIES

Chapter VII

PHYSICAL PROPERTIES OF STARCH

By Dexter French

1. Introduction. Within the past few years, new and improved methods of isolating starch components have induced a considerable renewal of interest in research on the structure of starch (1). The older starch "fractions" were poorly characterized and often highly degraded from the natural state. Previously, there had been a considerable diversity of opinion on just what factor or factors were responsible for the differentiation of starch fractions; some believed starch could be separated into its components by making use of differences in solubility; others believed the relative presence or absence of phosphorus or other inorganic constituents (impurities, perhaps) influenced the properties of the basic starch substance.

In the main, these older views have been eliminated, and at the present time an attempt is being made to ascribe the properties of starch or any of its fractions to the basic starch chain or its ramifications. On the whole, two extremes of starch types are recognized: amylose, simple straight chain starch (analogous to cellulose in chemical structure), and amylopectin, in which the regularities of the fundamental starch chain are interrupted by frequent branching. The structure of glycogen (animal starch) is similar, but more highly ramified.

Clearly, attempts to investigate the fundamental behavior of starch in any chemical or physical system should be made on homogeneous constituents rather than the whole starch. However, for lack of homogeneous starch preparations, much of the older literature was confused and even contradictory. The availability of chemically homogeneous starch components for physical examination not only facilitates research, but also renders the results reported less open to question. Although much painstaking research on the physical properties of starch has been carried out, much of this must be repeated on the homogeneous starch fractions before it can have any fundamental significance. We are therefore just beginning to build up a reliable body of literature on the physical behavior of starch substance.

With the perfection of fractionating procedures which separate sharply the amylose and amylopectin components of starches, it has become increasingly apparent that the individual fractions, though homogeneous with regard to chemical type, are nonetheless still heterogeneous with regard to such factors as molecular size, degree of branching, order or randomness of branching and other subtler features depending on the biological, physical or chemical history of the material (2).

2. Physical Properties of Native Starch Granules. Under the polarizing miscroscope, starch granules appear as somewhat distorted spherocrystals (3). The typical birefringence may be accounted for either on the basis of an actual crystal structure or on the basis of some other orderly radial or tangential distribution of starch chains. Frey-Wyssling (4) has measured the birefringence in some starch granules; he points out that the positive sign of birefringence (*i.e.,* with respect to the radius of the spherule) indicates a radial distribution of extended chains rather than coiled helices as suggested by Hanes (5) and Freudenberg (6). The magnitude of the birefringence (0.015) may be compared with that of cellulose (0.067). When leached with dilute sulfuric acid for several months, approximately 50% of the starch granule is dissolved (3). However, the resulting modified starch granule is as highly birefringent as ever, indicating that very little, if any, of the crystalline material is destroyed by this treatment. Furthermore, the crystalline residue must have a birefringence roughly twice that of the average of the whole granule. The starch product resulting from such prolonged hydrolysis *in the granule* has been found to consist almost entirely of an unusually homogeneous crystallizable compound of low molecular weight called amylodextrin (3, 7, 8). Amylodextrin spherocrystals resemble starch granules closely, and the X-ray diagrams are indistinguishable from those of the best undegraded starch preparations. Chemically, amylodextrin thus prepared is a chain of approximately 25 glucose residues, linked with typical starch unions, and containing very little or no branching. It may be inferred from these facts that the amylodextrin molecules (or residues) make up the crystalline regions in starch; the crystallites are connected by heterogeneous regions of non-crystallinity which are more susceptible to the action of the hydrolyzing acid, while the organized crystallites resist hydrolysis.* It does not follow that discontinuities in the starch molecules necessarily coincide with the crystallite boundaries, but rather that any one starch molecule may partake in several crystallites simultaneously, with any irregularities in the molecule lying in the amorphous regions.

The foregoing structure is supported by the physical behavior of starch granules on grinding or other types of granule mutilation such as strong desiccation. Microscopically, the effect can easily be observed by spreading a few starch granules on a slide and applying pressure to the cover-glass. The granules are seen to become distorted and lose their birefringence immediately; similarly, the X-ray diagram of physically mutilated granules becomes entirely amorphous in character. In other words, it is possible to

---

*The behavior of cellulose on acid treatment is indicative of a high degree of resistance to hydrolysis within crystallites, as compared with amorphous regions (9, 10).

"grind out" the crystallinity of starch without affecting its chemical properties significantly.†

This behavior is in marked contrast to that of ordinary crystalline compounds, such as sodium chloride or sucrose, which maintain their crystalline structure through similar treatment. It indicates that application of physical stress to the granule causes a disarrangement of molecules with a resulting breakdown of crystallinity rather than merely breaking the crystals apart, for in no case do the bulk of the particles obtained on grinding approach the size of the ultimate crystallites.*

This, in turn, is evidence that the crystallites are bound to each other by forces stronger than the forces between adjacent polysaccharide chains within crystallites. It is difficult to conceive of a type of bonding other than the polysaccharide covalent bond chain which would be sufficiently strong to withstand or even to lead to crystallite destruction.

The submicroscopic crystal structure of the starch granule bears little or no relationship to the source or type of starch. Starch granules from corn and waxy maize, which represent extremes of starch types, can be distinguished under the microscope only by means of the iodine reaction; furthermore, their X-ray patterns are only slightly different (11). In fact, starches from all sources, natural and synthetic, with the exception of glycogen, can be recrystallized to give crystal X-ray patterns which are identical with each other (12, 13).

3. Crystalline Forms of Starch

a. A and B Configurations. Two main types of crystal structure have been found in native starch granules. These two extremes have been designated "A," represented by cereal starches, and "B" represented by the tuber starches, while types intermediate between the extremes have been called "C" types (12).

On crystallization of aqueous starch pastes from any source, A, B, or C types of crystallization can readily be obtained, depending on the temperature of preparation (13). In general, the B type of crystallization is obtained by evaporation of pastes at room temperature or by precipitation of insoluble starch by freezing or retrogradation, the C type at a temperature somewhat above room temperature, and the A type up to about 80-90°C. At higher temperatures amorphous preparations are usually obtained. The crystal unit cell parameters of the A and B types suggested by Bear and French are (11):

---

†However, if grinding is continued long, the molecules are reduced in size, and the reducing power increases noticeably.

*On the basis of the width of X-ray diffraction lines, the crystallites have been estimated to be 100 A in size. This is roughly one-fiftieth of the visual limit. A fully extended starch chain 100 A in length would contain approximately 20 glucose units.

Type	a_0(A.)	b_0(A.)	c_0(A.)	α	β	γ	V(cu.A.)
"A"	15.4	8.87	6.18	87.0°	86.9°	92.8°	843
"B"	16.1	9.11	6.34	90.0°	90.0°	90.0°	930

A study of oriented fibers and films by Rundle *et al.* (14) partially confirms the crystal unit cell parameters of the B type configuration given by Bear and French. Two of the original dimensions appear to be correct but the dimension along the molecular axis is apparently 10.6 A. Table XI gives a comparison of the unit cell dimensions of starch, maltose, cellulose, and cellobiose. The orientation of the molecules in maltose, cellulose, and cellobiose may be determined by the optical properties of the crystals or fibers and in each case the dimension closest to 10 A. represents the length of the biose molecule or residue. The same conclusion applies to starch. However, the reason that the length of the primitive translation for starch should be greater than for cellulose is not readily apparent. Possibly the pyranose rings in starch are distorted, or possibly the α-glycoside linkage is sufficiently flexible to allow the formation of a longer unit cell.

TABLE XI

	a_0(A.)	b_0(A.)	c_0(A.)	β
Starch	16.0	10.6	9.2	90°
Maltose (hydrate) (8)	10.7	15.2	4.9	82.5°
Cellulose, native	8.35	10.3	7.95	84°
Cellobiose	11.1	13.2	5.00	90°

The structure of B starch suggested by Rundle, *et al.* is given in Figure 52.

b. V Type Configuration. When starch pastes are precipitated with alcohols or some other precipitating agents, an entirely different type of crystallization takes place (7). The X-ray diffraction pattern of alcohol-precipitated starch ("V" pattern*) is much simpler than the A or B type of pattern, indicating a more symmetrical arrangement of molecules, and starch thus precipitated differs notably from granular starch in other respects (15). Freshly precipitated starch of the V type is readily dispersible, often in cold water; on standing the material gradually loses its solubility in water, and, if allowed to stand moist, retrogrades to the B configuration. A most striking singularity of alcohol-precipitated starch, *i.e.*, starch of the V configuration, is its ability to take up large amounts of iodine vapor (8, 16)

---

*Verkleisterungspektrum, or paste pattern.

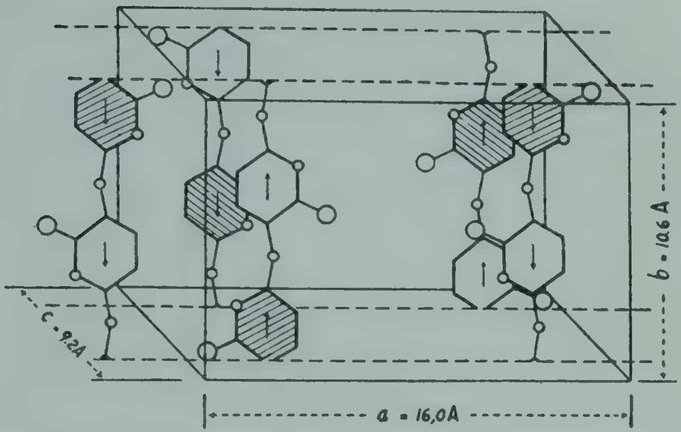


FIG. 52. Structure of the B-modification of starch. (Rundle, Daasch and French, *J. Am. Chem. Soc.*, **66**, 133, 1944.)

Starch pastes precipitated with an excess of ethanol or propanol are coarse and formless, and the V pattern consists of but three or four more or less sharp lines. When butanol or amyl alcohol is used, a fine crystalline precipitate slowly forms (17), and the X-ray pattern is relatively rich. However, when the precipitate is dried, the simple V pattern is obtained. Starch precipitated with tertiary butyl alcohol gives an X-ray pattern similar in appearance to the ordinary simple V pattern, but the ring diameters are somewhat smaller, indicating a larger lattice. Starch precipitated with iodine and potassium iodide gives a fair V type of pattern (15).

The X-ray pattern of starch of the V type which has absorbed about 20% of its weight of iodine vapor is a simple appearing pattern bearing no relationship to the iodine crystal pattern but resembling strongly the V pattern. On calculation of the interplanar spacings, it is found that all strong diffractions can be accounted for on the basis of a two-dimensional pseudo-hexagonal reciprocal lattice, indicating a molecular arrangement like that of closely packed cylinders 13 A. in diameter (16).

Rundle and Edwards (18) succeeded in analyzing the crystal structure of butanol precipitated amylose by combining information from samples which were dry, saturated with wet alcohol, or saturated with iodine. They were able to show that V-amylose crystallizes in a pseudo-hexagonal orthorhombic unit cell with $a_0 = 13.7$ A., $b_0 \doteq 25.6$ A., and $c_0 = 7.8$ A. when wet, which changed on drying to $a_0 = 13.7$ A., $b_0 = 24.8$ A., $c_0 = 8.05$ A. The structure is best interpreted as consisting of helical tubes 13 A. in diameter, of indefinite length, packed together in pseudo-hexagonal fashion. On drying, the overall aspect of the arrangement is maintained but it becomes somewhat less highly ordered, the amylose helices pack together more closely and the diffraction pattern is rendered less sharp.

Electron density projections have been carried out by Rundle (19) for V-amylose and the amylose iodine complex; the helical configuration is clearly evident (Figs. 53 and 54).

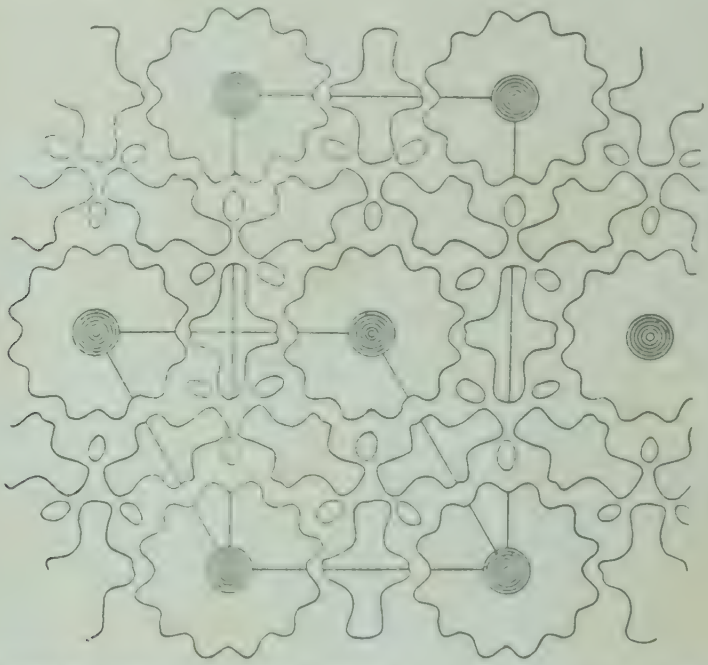


Fig. 53. Fourier projection of the amylose-iodine complex. (Rundle, *J. Am. Chem. Soc.*, **69**, 1771, 1947).

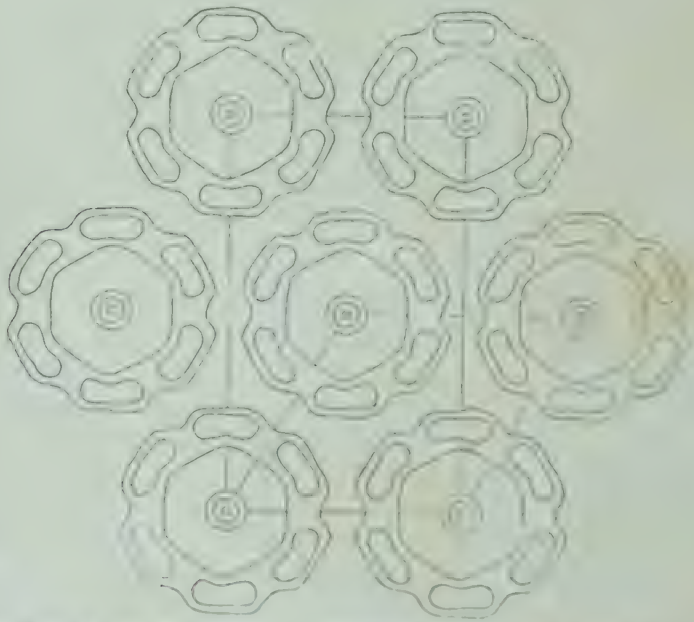


Fig. 54. Fourier projection of V (butanol-precipitates) amylose. (Rundle, *J. Am. Chem. Soc.*, **69**, 1772, 1947.)

The fact that granular starch is in an extended configuration has led some investigators to discount earlier evidence for a helical starch molecule. However, this earlier evidence was based on the behavior of starch in solution, and need not bear any relationship to the configuration of solid crystalline starch. Space-filling helical models of amylose molecules are approximately 13 A. in diameter, in agreement with X-ray results.

c. Crystalline Amylose. Crystals of butanol-precipitated amylose are quite birefringent under the polarizing microscope, although not to the same extent as granular starch (20). Under certain conditions of crystallization, the crystals are more or less rectangular platelets which are pseudouniaxial and birefringent negative, the pseudooptic axis being normal to the platelet surface. On treatment with very dilute iodine solution, the crystals take up iodine readily and become extremely dichroic, indicating that the iodine molecules are highly oriented within the crystals. Since light having its electric vector normal to the platelet surface is extinguished while all other light passes practically without absorption, the iodine molecules must lie normal to the platelet surface.* The only structure consistent with the foregoing properties is an arrangement of helical starch molecules oriented with the helical axis normal to the platelet surface. The iodine molecules taken up by the amylose lie on the helix axis and are held there rigidly oriented normal to the surface (21, 16).

As it first comes down, the total butanol-precipitated fraction of starch is in the form of six-sided rosettes which are probably aggregates of rectangular platelets. The rosettes have the same characteristic optical properties before and after treatment with iodine, and the X-ray diffraction patterns are identical.

d. Amylose Films. Hot solutions of starch, and especially of amylose, tend to form strong insoluble surface skins which can be removed and washed readily (17). These films are strongly birefringent, even when soaking wet, and are uniaxial negative with respect to the film surface normal (8). In converging polarized light these films exhibt a typical but weak uniaxial negative interference figure. The starch chains are evidently grouped parallel to the film surface, judging from the optical characteristics and the fact that the direction of most rapid increase in size of a growing crystal or crystalline aggregate is usually normal to the long axis of the molecule which, in the case of the film, is also normal to the film surface. From amylose films are obtained oriented X-ray diffraction patterns which are of value in the interpretation of the crystal structure of starch (14). Moreover, with oriented films it is possible to examine the degree of swelling on treatment with swelling agents in directions parallel and normal to the amylose chains. On treatment with dilute iodine solutions, the films become dark blue or black, but not noticeably dichroic.

---

*Iodine molecules absorb only light having the electric vector parallel to the axis of the molecule.

c. Amylose Fibers. By deacetylation of amylose acetate fibers while held under tension, Senti and Witnauer (22) have prepared almost perfectly oriented fibers of amylose and several amylose derivatives. For alkali amylose a fiber X-ray diffraction was obtained (Fig. 55) with a degree of perfection seldom attained even in the best natural fibers. Alkali amylose has an orthorhombic unit cell with $a_0 = 9.0$ A., $b_0 = 22.7$ A., $c_0 = 12.7$ A., containing 12 glucose residues per unit cell.

Transformation of alkali amylose fibers into V-amylose fibers was accomplished by soaking in 75% methanol or other primary alcohols. The fiber spacing for V-amylose was 8 A., in agreement with the helix periodicity found from powder patterns (16, 18); lateral spacings were found to be dependent on the moisture content. *Tert.* butyl alcohol produces a similar fiber with 7.9 A. fiber periodicity but the lateral reflections were considerably altered indicating a monoclinic arrangement.

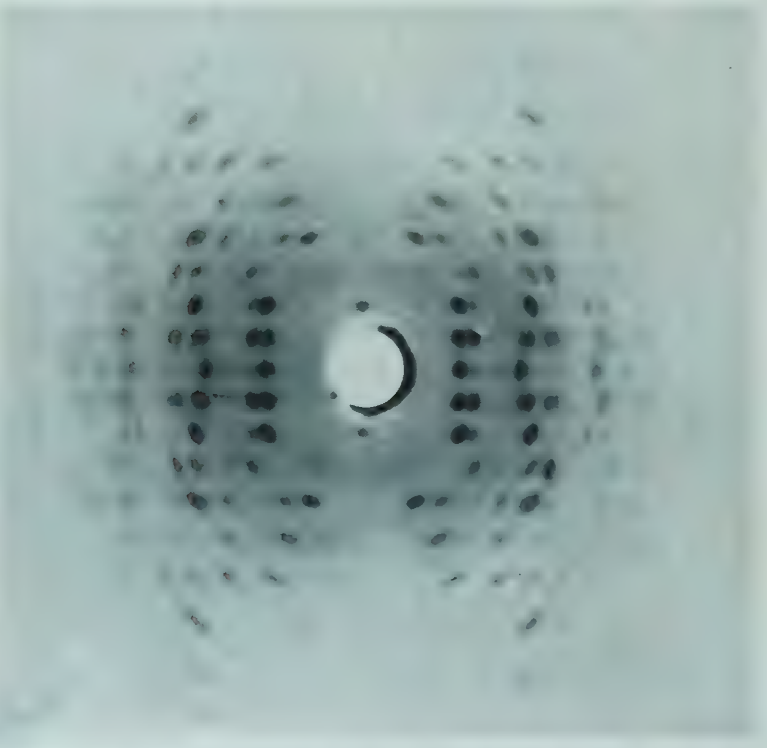


FIG. 55. Fiber diffraction pattern of alkali amylose. (Senti and Witnauer, *J. Am. Chem. Soc.*, **68**, 2408, 1946.)

The perfection of Senti's amylose fibers together with the wide variety of complexes which crystallize as amylose fibers offer interesting possibilities for more detailed structure analysis.

4. Swelling, Gelatinization, and Retrogradation of Starch. The breakdown of the structure of the starch granule on heating in water takes place in three quite distinct phases. During the first phase, water is slowly and reversibly taken up, and limited swelling occurs. The viscosity of the suspension does not increase noticeably. The granule retains its characteristic appearance and bifringence, and upon cooling and drying, no obvious changes can be observed. Within a small range of temperature at approximately 65°C. (the exact temperature being a characteristic of the variety of starch) the second phase of swelling starts; the granule suddenly swells, increasing many times in size, taking up a great deal of moisture and rapidly losing its birefringence. The second phase of swelling is also marked by a rapid rise in the viscosity of the starch suspension, and upon cooling the granules are altered in appearance, most of them having lost their structure and birefringence. A small amount of the starch has become solubilized, as can be shown by treating the supernatant liquid or centrifugate with dilute iodine solution. During the third phase of swelling, which takes place with increasing temperatures, the granules become almost formless sacs, and the more soluble part of the starch is leached out. Suspensions containing only a few per cent of starch are so filled with the swollen granule sacs that on cooling a rigid gel is formed.

The exact mechanism of swelling, the second phase of which may be said to be the active phase, is somewhat obscure in spite of much investigation and speculation. The swelling may be induced at room temperature by many chemical agents, notably alkali and metallic salts. By controlling the concentration of these agents, it is possible to reduce the speed with which the second phase of swelling takes place for convenience in microscopic examination.

By using swelling agents, one can observe the formation of a bubble in the interior of the granule during the second phase of swelling (23). This bubble grows in size until the granule becomes weakened and the bubble rapidly diminishes and usually entirely disappears. This phenomenon indicates that during the rapid swelling process the interior of the granule is a region of very low pressure, so much so that a void is formed and expands at the pressure of water within the system. The forces producing swelling must be largely in a direction tangent to the granule surface, since a radial expansion would not reduce the pressure in the interior of the granule.

Some insight into the nature of the swelling forces may be gained through the observation of the swelling of oriented films in which the molecules lie parallel to the film surface (8). On treatment with swelling agents, a film increases approximately 50% in the surface area, while the thickness increases to about three to five times its original value. The only interpretations of these facts which is consistent with the physical structure of the amylose film is that the amylose molecule swells in a direction normal to the axis of the molecule without any decrease in length. Since the amylose film is constrained by the molecular valence forces of the extremely long amylose chains, the bulk of the swelling must occur in a direction normal to the film surface. This direction is approximately normal to all the molecular chains, and there can be no constraint to the swelling. When swelling takes place normal to the starch molecules in the granule, it produces the observed tangential expansion and lowering of the interior pressure.*

Starch granules which have been solubilized by the action of acids are no longer capable of swelling, but on treatment with hot water the granules disintegrate and pass into solution. The solubilization breaks the starch down to molecules of smaller size which are incapable of forming the giant networks characteristic of the swollen granules.

Dilute pastes consisting largely of swollen granules may be thinned to a large extent by boiling or other mechanical treatment, such as passing through a homogenizer. Such dilute pastes have less tendency to set rapidly to strong gels, but at higher concentrations gels are readily formed, even from thoroughly dispersed starch. These more concentrated gels depend for their rigidity on the interlacing of the starch molecules, especially the amylose molecules with straight chains. In this connection it is significant that the highly branched glycogen cannot be induced to gelatinize or crystallize, while amylose forms gels in relatively dilute solutions.

Solutions of starch which have aged at room or lower temperature undergo the phenomenon of retrogradation. A part of the starch aggregates progressively, and finally forms an insoluble microcrystalline precipitate.**

The aggregation of starch makes it unavailable to the action of enzymes, even though the aggregates remain "dissolved"; *i.e.*, they do not precipitate out. The retrogradation process may be hastened by freezing aqueous solutions; in this way, ordinarily stable solutions may be forced to retrograde. Although some amylopectin preparations have a tendency to retrograde from solution, the property is greatly exaggerated in pure amylose solutions. Even 1% solutions of corn amylose will deposit practically the whole amount of solute in the form of crystalline sediment in the course of a few days.

---

*In distinction to amylose films, when collagen (untanned leather, etc.) films are treated with hot water, within a fairly narrow temperature range the collagen swells markedly in thickness but at the same time undergoes considerable shrinkage in length and surface. The characteristic temperature is known to the leather trade as the shrink temperature, and the phenomenon is probably due to the coiling or folding of the protein chains as they are released from the original semicrystalline structure under the influence of heat and moisture.

**The crystallinity of retrograded starch is demonstrated by the fact that it gives a crystalline X-ray pattern of the B type. Under some conditions retrograded amylose is noticeably birefringent.

The process of retrogradation takes place even in the solid state. The staling of bread is such a process (24), and starches precipitated by alcohol and left moist readily retrograde. Retrogradation is arrested by swelling agents, by keeping preparations above room temperature, or by removing the moisture.

5. Fractionation of Starch and Properties of Starch Fractions. The multiple amylose theory of starch composition rests in the main on efficient methods of fractionating starch into its ultimate components. Quite recently new and improved methods of fractionation have been developed which allow the isolation of undegraded starch components which are basically different in structure and have quite different physical properties. It has also been possible to synthesize *in vitro* types of homogeneous starch whose properties are practically identical with natural starch fractions. According to Meyer (1), *amylose* is used to designate unbranched starch chains, while *amylopectin* represents the component of starch that has branched chains. It must be noted that these definitions are on the basis of chemical structure, especially with respect to the presence or absence of branch linkages, while the older definitions were on the basis of such properties as solubility, enzyme digestibility, iodine color, etc. Although it is true that many other criteria may be used to distinguish between amylose and amylopectin, ultimately the burden of proof of structure of a starch fraction must fall on painstaking chemical analysis. Meanwhile, in exploratory work, we must use simpler techniques, such as (1) enzyme digestion (β-amylase), (2) crystallization methods, (3) adsorption on cellulose or other adsorbents, (4) determination of reducing power or alkali lability (aldehyde assay), (5) iodine absorption spectrum, (6) potentiometric iodine titration, (7) X-ray diffraction, and (8) viscosity and allied methods.

a. Hot Water Fractionation. By extracting swollen corn starch granules with hot water, Meyer found that 5 to 20% of the total starch could be leached out, and that on concentration the extract became insoluble (25). By chemical analysis of end-groups, the material was shown to be fairly pure amylose, *i.e.*, starch with straight chains; the granule residues were shown to contain the branched molecules originally present in the whole starch.*

The mechanism involved in fractionation by hot water is the loosening of the granule structure and the extraction of molecules not too tightly bound or entangled in the network. The amylose molecules are able to diffuse slowly out of the swollen granules, while branching in the amylopectin molecules tends to cut down the rate of diffusion to practically zero. It is reasonable to suppose that the fractionation will not be a complete one since some of the straight chain amylose molecules will be so hopelessly entangled that their rate of diffusion out of the structure will be very slow; on the other hand, many of the amylopectin molecules on the surface of the granule will be torn loose and will appear as an impurity in the amylose. For these reasons, it is advisable to purify the raw amylose by another fractionation technique.

b. Selective Alcohol Precipitation. A fundamentally new method of starch fractionation was discovered by Schoch during an investigation of the effect of alcohols on starch pastes (17). It was found that butanol and Pentanol cause a selective precipitation of the amylose constituent of starch, leaving the amylopectin in solution (26). Although the raw amylose contains a considerable amount of coprecipitated amylopectin, by repeated crystallization the amylose becomes substantially free from branched chains. The non-precipitated fraction contains a small amount of amylose which gives this fraction a deep blue iodine color; however, on the removal of less than 10% of the material by adsorption on cotton (see below) the blue-staining characteristics are removed and pure amylopectin remains.

X-ray and optical analysis of the butanol precipitate has shown that the amylose chains form helical coils, presumably around the alcohol molecules, and the cylindrical molecules then pack closely with the formation of the characteristic rectangular platelets and hexagonal rosettes of the butanol precipitate. In amylopectin the frequent branching interrupts the regularity of the amylose chain, and the molecule is unable to participate in the helical, cylindrical crystallization. The molecular weight of butanol-precipitated amylose is considerably higher than that of the corresponding amylose from hot water extraction (20); possibly this is because of the presence of an impurity of high molecular weight, but more likely because in the hot water extraction the amylose of high molecular weight is more slowly extracted because of its higher entanglement in the granule structure. Moreover, an impurity of very high molecular weight, *i.e.*, amylopectin, would have little effect on the *average* molecular weight of the amylose preparation.

---

*However, Meyer did not show that all the amylose had been extracted from the insoluble granule residues, and since higher yields of amylose have been obtained by other methods, it is most likely that the amylopectin fraction obtained by this procedure is contaminated with amylose.

The technique of butanol precipitation is one of the most important recent advances in carbohydrate chemistry. It can be used by itself or to supplement other methods of fractionation; in each case, a fairly clean cut separation is obtained and the ease of recrystallization of the precipitate makes it probably the most practical of the fractionation techniques.

c. Cellulose Adsorption. The amylose constituent of starch mixtures is strongly adsorbed on cotton or filter paper (20, 27). By this technique, it is possible to prepare amylopectin practically free from amylose, and also amylopectins prepared by other methods may be conveniently separated from their amylose impurity. The amylose can be removed from the cellulose by extraction with boiling water. The method was discovered long ago by Tanret and has recently been revived by Pacsu.

d. General Properties of Starch Fractions. Some of the distinguishing properties of amylose are (1) high digestibility with β-amylase (practically 100%), (2) relatively low molecular weight (the molecule contains a few hundred glucose residues with only one non-reducing end-group per molecule), (3) pure deep blue color with iodine, (4) absorption of large amounts of iodine isopotentially in potentiometric titration, (5) practically complete crystallizability with butanol or other crystallizing agents, (6) complete adsorption on cellulose, (7) high intrinsic viscosity and low solution stability at ordinary concentrations in water.

The corresponding properties of amylopectin are (1) lower digestibility with β-amylase (about 50%), (2) high molecular weight (the molecule contains over 1000 glucose residues with one non-reducing end-group for every 20 to 30 glucose residues), (3) purple or reddish color with iodine, (4) does not absorb iodine isopotentially, (5) does not form a crystalline complex with butanol but gives A or B and V type patterns of poor crystallinity, (6) unadsorbed on cellulose, (7) fairly high solution stability, *i.e.*, low tendency to retrograde.

e. Properties of Synthetic Starches. Both plant and animal phosphorylases carry out the synthesis of amylose-like polysaccharides (28, 29) from Cori ester and a suitable co-substrate (glycogen, dextrins, etc.). These synthetic amyloses have many properties closely resembling natural amyloses: low solution stability and high tendency to precipitate or retrograde, formation of a blue-black insoluble iodine complex, absorption of large amounts of iodine in the potentiometric titration, relatively high molecular weight, and the production of characteristic amylose X-ray diffraction patterns (29, 30).

In the presence of an enzymatic branching factor ("Q enzyme") the product synthesized by phosphorylase resembles glycogen (29) or amylopectin (31). With large amounts of dextrin co-substrate, amylodextrin-like polysaccharides are formed (32).

6. Iodine Reaction with Starch

a. Mechanism of Iodine Reaction. Since the discovery of iodine, it has been known that starch is capable of reacting with iodine to give a deep blue color, and there have been many investigations and speculations on the nature of this reaction. It has not been understood until very recently whether a stoichiometric reaction is involved, whether iodide ions or other anions are necessary, or if water is essential to the formation of the starch-iodine complex.

On the basis of the iodine coloration and the molecular weights of dextrins of α-amylase, Hanes postulated a helical structure for starch and the starch-iodine complex. He found that dextrins having less than 6 glucose residues per molecule are uncolored by iodine solution, and he attributed this to the inability of the dextrins to form a complete turn in a helix (5).

This mechanism was given support by Freudenberg, who constructed models of the starch helix and the closely related cycloamyloses (Schardinger dextrins), and showed that these molecules have hydrocarbon-like linings (free from the fields of the strongly polar hydroxyl groups) in which the iodine could assume the color characteristic of its solutions in non-polar solvents. The fact that the cycloamyloses form well crystallized iodine addition products, while the linear amylodextrins of corresponding molecular weight do not react to form colored complexes with iodine, was also considered strong evidence that a ring or helical molecule is required for the characteristic iodine reaction (6).

Since many colloidal precipitates and some simple cyclic acetals react with iodine to give colored complexes somewhat similar to starch-iodine, the helical concept of the starch-iodine reaction has not met with complete approval. Meyer believes that all the facts concerning the reaction can be accounted for by the adsorption of iodine at the interstices of the crystallites (33). Recently, however, new facts concerning the starch-iodine reactions have given convincing support to the helical model.

As mentioned in an earlier section, amylose in the helical V configuration is capable of absorbing large amounts, up to 25% or more by weight, of iodine. The reaction takes place as readily when dry amylose is treated with iodine vapor as when amylose solutions are treated with dilute iodine solutions. The helical configuration is essential since other configurations are incapable of absorbing a significant quantity of iodine in the absence of moisture.

b. Potentiometric Titration of Starch with Iodine. The nature of the reaction between starch or starch fractions and iodine has been investigated by analyzing the precipitated complexes and by following the optical absorption curves after the addition of iodine, but the results of these analyses have sometimes been contradictory and often misleading. A more illuminating method is to follow the reaction potentiometrically, while the iodide ion concentration is kept constant (30). The activity of free iodine in a solution is readily determined potentiometrically, and it is then a simple matter to determine how much iodine is bound by starch at various iodine levels. On the addition of iodine to an amylose solution in 0.05 *N* potassium iodide, the iodide activity rapidly approaches a value which remains constant until iodine equal in weight to approximately 18.7% of the amylose has been bound. This is about 1 iodine molecule per 8 glucose residues. At this point, the iodine activity rises more rapidly on the addition of iodine solution, however, not as fast as in potassium iodide solution alone. This rather abrupt change in the rate of increase of iodine activity at 18.7% absorption, followed by additional absorption, indicates beyond doubt that there are two distinct phases of the reaction: (1) Iodine is taken up isopotentially, combining with the amylose at a potential which is characteristic for the type of amylose present; and (2) increasing amounts of iodine are taken up at increasing iodine potentials. The first phase, during which the typical blue color is formed and gradually deepened as iodine is added, has many of the characteristics of a heterogeneous reaction; that is, the iodine activity is a constant independent of the amount of either amylose or the iodine addition product present. Following 18.7% absorption, the characteristics of the reaction indicate absorption at the surface of the molecules or the distribution of iodine between two immiscible solvents.

The potential at which iodine is taken up during the titration of an amylose or other starch fraction is a characteristic of the sample and is apparently a function of the chain length. Thus, corn amylose, which has a molecular size of approximately 250 glucose residues, takes up iodine at a somewhat higher potential than does potato amylose, the molecules of which are roughly twice as large. On the other hand, amylodextrins, short straight chain amyloses of about 20 to 30 glucose residues, take up iodine at a much higher potential. In mixtures, the constituent which absorbs iodine at the lowest potential, is titrated first, then the potential rises abruptly to the next lowest potential, and so on. This behavior is characteristic of heterogeneous systems.

Amylopectin fractions and glycogen do not take up iodine isopotentially, but at gradually increasing potentials somewhat as in the second phase of the amylose reaction. Moreover, the amount of iodine bound is much smaller than in amylose. Instead of a deep blue color, a reddish or brown color is developed, as is also the case with the amylodextrins of low molecular weight. Raw starch or mixtures of amylose and amylopectin may be analyzed by potentiometric titration; the absorption of iodine by the amylose takes place as if no amylopectin were present, and the amount of amylose can be readily calculated on the basis of 18.7% iodine for pure amylose.

The behavior of whole starch during potentiometric titration indicate the presence of but two distinct fractions, amylose and amylopectin. All so-called fractions which have properties intermediate between amylose and amylopectin are titrated as mixtures of amylose and amylopectin, and in most cases subfractionation has been successful. The potentiometric titration of starch with iodine then constitutes a powerful argument in favor of a two component theory for starch, as well as a useful tool for the analysis o whole starch and starch fractions.

c. Optical Properties of Amylose-Iodine Complexes. Extremely dilute amylose-iodine solutions exhibit very broad absorption bands with maximum absorption at about 600 mμ, while with amylopectin or amylodextrin solutions the absorption maxima are shifted to shorter wavelengths (34) (see Fig. 56). The absorption in starch-iodine solutions is much more intense than that that in solutions containing corresponding amounts of iodine in organic solvents (35).

Amylose-iodine solutions oriented during flow become quite dichroic indicating an orientation of the iodine molecules (36). Since the velocity

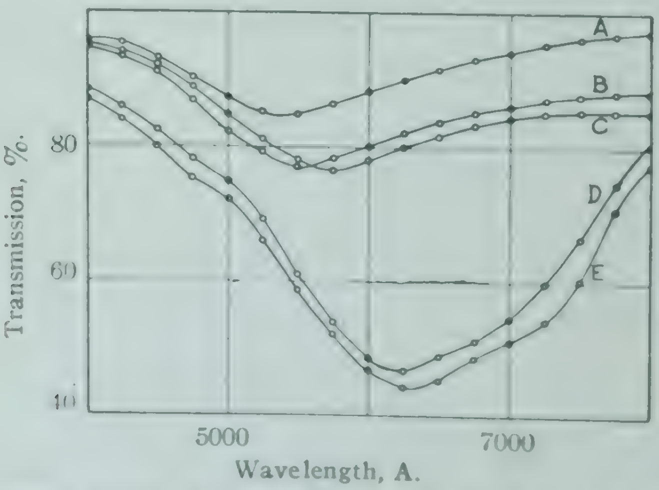


FIG. 56. Transmission curves of the iodine complexes of amylose and amylopecti materials: *A*, waxy maize; *B*, potato amylopectin; *C*, corn amylopectin; *D*, corn amylose; *E*, potato amylose; cell depth, 1.63 mm; iodine conc. 5×10^{-5} *M*; starch conc. 0.01%. (Baldwin, Bear and Rundle, *J. Am. Chem. Soc.*, **66**, 112, 1944.)
]

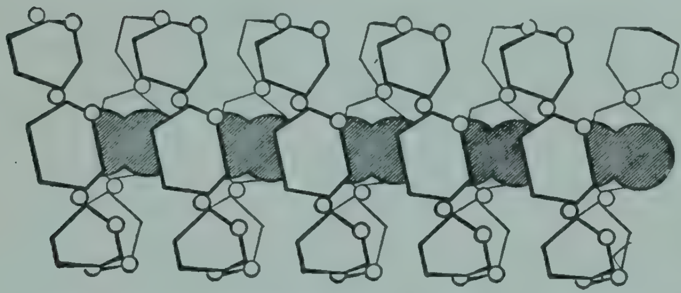


FIG. 57. Model of an iodine-filled amylose helix. (Rundle, Foster and Baldwin, *J. Am. Chem. Soc.*, **66**, 2117, 1944.)

gradients in streaming fluids would scarcely orient iodine molecules, one must assume that the amylose molecules become oriented and in turn orient the iodine molecules. The dichroism indicates that the long axes of the iodine molecules are parallel to the direction of flow, while the highly asymmetric amylose molecules must also be oriented roughly parallel to the flow lines. These considerations, together with evidence from X-ray diffraction, indicate that in amylose-iodine solutions as well as amylose-iodine crystals the iodine molecules are tightly held in the center of a helical amylose coil (see Fig. 57).

During flow, the helices orient themselves roughly parallel to the flow lines and thereby orient the iodine molecules.

On precipitation of starch-iodine, the helices pack together closely to give an organization which exhibits a V type X-ray pattern (15). The iodine molecules must be packed practically end to end if there is one iodine molecule for every 6 or 8 glucose residues. It is questionable whether a regular periodicity exists or whether the iodine molecules are packed more or less at random, but at any rate the molecules are held rigidly parallel to the helix axis. The intense light absorption, characteristic of the amylose-iodine complex, is due to the cooperative effect of many collinear iodine molecules (37).

7. **Optical Rotation of Starch.** Most values for the specific rotation of starch range between $[\alpha]_D = +180$ and 220°; in fact, there are so many different values in the literature that one is tempted to believe that each worker has his own method for determining the specific rotation of starch fractions. On the other hand, most values reported for hydrolytic dextrins of high molecular weight which are readily soluble lie between $[\alpha]_D = +190$ and 200°. The difficulty in measuring the rotation of undegraded starch arises from the opacity of the solutions used. For this reason it is the custom of many workers to measure the rotation of starch in alkaline solutions which are relatively clear. By dissolving amylose in alkali, neutralizing, and

immediately taking the rotation, Meyer obtained a value of $[\alpha]_D = 220 \pm 5°$ (1). This value is significantly higher than the average of values reported by other workers for whole starch. The specific rotation of amylopectin may be taken as $[\alpha]_D = +200°$; however, the high opalescence and opacity of aqueous solutions make his value somewhat uncertain. On the other hand, freshly prepared solutions of butanol-precipitated amylose give clear limpid solutions whose rotations can be measured almost as accurately as those of ordinary crystalline compounds. Using clear supernatants from freshly prepared amylose solutions, one obtains values of $[\alpha]_D = +200°$ with an uncertainty of about 2°. The butanol-precipitated amylose contains a large amount of water and butanol; so it is impossible to make up the solutions accurately by weight, and there are usually some particles of amylose which are undissolved even after boiling several minutes. For these reasons, it is necessary to determine the concentration of the solution used for the measurement of specific rotation by evaporating a small amount to dryness.

Since it is relatively simple to determine accurately the specific rotation of amylodextrins, as these compounds give water-clear solutions, it is possible to calculate a value for the specific rotation of an amylose of infinite chain length and hence that of an amylose of any chain length by means of the so-called Freudenberg equation (38).

$$[M]_n = [M]_2 + (n-2)\,[M]_\infty/\infty$$

where n is the number of glucose residues in the amylose chain, $[M]_n$ and $[M]_2$ are the molecular rotations of the n-membered amylose and maltose respectively, and $[M]_\infty/\infty$ is the molecular rotation per glucose residue of an infinitely long amylose chain. For example, an amylodextrin containing 22 glucose residues has a specific rotation of $[\alpha]_D = +193°$, while maltose hydrate has a rotation of $[\alpha]_D = +131°$. The corresponding molecular rotations are then $[M]_{22} = 691{,}300$ and $[M]_2 = 47{,}200$, and the molecular rotation per glucose residue is $[M]_\infty/\infty = 32{,}200$. This corresponds to a specific rotation for the infinitely long amylose of 199°, which is in excellent agreement with the above value obtained from butanol-precipitated amylose.

As the value of the specific rotation of amylopectin is also very close to $[\alpha]_D = 200°$, small amounts of amylopectin will not influence the value obtained for the specific rotation of amylose. Since amylopectin has an irregular structure and is rather difficult to work with in the polarimeter, comparatively little is known about the amylopectin dextrins. For the present, one may assume that amylopectin dextrins have roughly the same specific rotation as the corresponding amylose dextrins.

Reeves has shown that starch and cellulose exhibit a striking rotatory shift when dissolved in cuprammonium hydroxide, amounting to approximately —1000° in the specific rotation in blue light. This effect is probably due to the *trans* configuration on carbons two and three of each individual glucose unit; similar rotatory shifts were observed with suitably substituted methyl glucoses (39).

8. Other Aspects of the Colloidal Behavior of Starch. The sizes and shapes of homogeneous colloidal molecules may be determined from the results of ultracentrifugal measurements together with data on the rate of diffusion, partial specific volume, and viscosity of both solution and solvent. As yet comparatively little has been published concerning these relatively important phases of the starch problem. A complete investigation of the sedimentation and diffusion of glycogen by Bridgman (40) indicates what may also be expected of homogeneous starch fractions. He found that five samples of glycogen were quite highly polydisperse, with average molecular weights ranging from 4 to 14 million (corresponding to molecules containing 25 to 90 thousand glucose residues). The experiments indicate an unexpectedly high asymmetry for glycogen, which is usually considered to be roughly spherical; indeed an axial ratio of 18:1, or higher, was found to exist.

An ultracentrifugal investigation of whole ground corn starch by Beckmann and Landis (41) indicates that the starch is decidedly heterogeneous and consists of two main fractions. The heavy component sediments very rapidly, having a sedimentation constant of approximately 6000 S, while the lighter component has an average sedimentation constant of 4 S with considerable spread of S values. Rough diffusion constants were also determined from the ultracentrifuge diagrams and were used to calculate molecular weights and asymmmetry ratios. These authors estimate the average molecular size of the amylose fraction to be approximately 300 to 400 glucose units, while the amylopectin fraction is thought to be about 1000 times larger. The axial ratio of amylose, estimated from the f_s/f_o values given, ranges from 2.5 (almost spherical) to about 45.

A similar investigation of potato starch by Coles (42) showed that potato amylose consists of molecules of higher average molecular weight and of considerably higher asymmetry than corn amylose. The average molecular weights given by Coles for potato amylose and amylopectin are 185,000 and 1,000,000 respectively. After degradation by grinding or enzyme action the molecules become much smaller and less asymmetric.

It must be pointed out that while it is possible to determine diffusion constants of homogeneous compounds of high molecular weight in the ultracentrifuge, the values obtained in this way from heterogeneous preparations and compounds with long molecular chains in particular are not generally acceptable. The difficulties of maintaining starch preparations, especially amylose, in aqueous solution without aggregation or degradation also complicate the problem.

Earlier sedimentation experiments on starch are now generally considered unreliable, inasmuch as the samples were mixtures of unknown proportions and moreover were often highly degraded. The difficulty of maintaining types of starch in a conditon of complete dispersion without degradation has been solved by the use of such solvents as chloral, hydrazine, ethylenediamine, and others; we may presently expect more reliable reports on the sedimentation and diffusion of starch components.

The high viscosity of completely dispersed starch solutions must be interpreted as a strong indication that the molecules are, on the average, highly asymmetric. On the basis of studies by Staudinger, average starch molecules are appreciably less asymmetric than cellulose molecules of the same molecular size (43). Although Staudinger did not have access to the newer starch fractions, it is also true that even the best amylose preparations do not cause as much increase in viscosity as corresponding cellulose solutions (44). This is due, in part, to the difference in the configuration of the glucosidic linkage and to the tendency of the starch chains to form helical coils, both of which cause a considerable shortening of the molecule.*

Studies of amylose acetates by Dombrow and Beckmann (45) lead to the conclusion that these substances dissolved in methyl acetate are considerably less asymmetric than cellulose acetates of equivalent molecular weight. Length-diameter ratios of about 40:80 were obtained from ultracentrifuge measurements; viscosity experiments indicated somewhat lower ratios of 26:42. From the ultracentrifuge results, these workers calculated an average length per glucose unit of about 2 A. in comparison with 5 A. for cellulose acetate.

Foster and Lepow (46) have carried out an extensive study on the birefringence of flow of various amyloses dissolved in ethylenediamine-glycerol mixtures. They were able to show that the amylose molecules at rest are more or less coiled up, but under the influence of a strong flow gradient they stretch out and become highly oriented. These effects are in marked contrast to the behavior of globular proteins, which behave as fairly rigid particles.

---

*All evidence indicates that amylose chains are much more flexible than cellulose chains. The ready conversion of amylose solutions to the linear arrangement in either A or B modifications, or to the helical V configuration; the ability of amylose chains to form closed rings (cycloamyloses) by enzymatic action; the ultra-centrifuge and viscosity results; and particularly the stretching effect observed in birefringence of flow experiments; all these point to a marked flexibility of the amylose chains and indicate in a very convincing manner the high degree of flexibility of amylose chains.

Investigations on the scattering of light by starch fractions are in progress; preliminary reports indicate that the molecular weights of amylopectins from various sources are at least several million (47). Molecular weight determinations by osmometry (44) indicate extremely high molecular weights for amylopectins but here again the experimental procedure is probably not sufficiently precise to give more than minimal molecular weights. Further, in determinations of solution molecular weights it is essential that the solute be completely dispersed, and yet undegraded. The difficulty of achieving this condition has undoubtedly been the largest single factor in retarding our knowledge of the molecular properties of native starch substance.

BIBLIOGRAPHY

1. Meyer, K. H. *Advances in Colloid Sci.*, **1**, 143 (1943); See also *Natural and Synthetic High Polymers*, **4**, 387 (1942).
2. Kerr, R. W., *Arch. Biochem.*, **7**, 377 (1945); *J. Am. Chem Soc.*, **67**, 2268 (1945).
3. Meyer, A., Untersuchungen über die Stärkekorner, Jena, G. Fischer (1895).
4. Frey-Wyssling, A., *Naturwissenschaften*, **28**, 78 (1940).
5. Hanes, C. S., *New Phytologist*, **36**, 189 (1937).
6. Freudenberg, K., *Naturwissenschaften*, **27**, 850 (1939).
7. Brown, H. T., and Morris, G. H., *J. Chem. Soc.*, **55**, 449 (1889).
8. French, D., Dissertation, Iowa State College, Ames, Iowa (1942).
9. Nickerson, R. F., and Habrle, J. A., *Ind. Eng. Chem.*, **39**, 1507 (1947).
10. Philipp, H. J., Nelson, M. L., and Ziifle, H. M., *Textile Research J.*, **17**, 585 (1947).
11. Bear, R. S., and French, D., *J. Am. Chem. Soc.*, **63**, 2298 (1941).
12. Katz, J. R., and van Itallie, T. B., *Z. physik. Chem.*, **A 150**, 90 (1930).
13. Katz, J. R., and Derksen, J. C., *Z. physik. Chem.*, **A 165**, 228 (1933).
14. Rundle, R. E., Daasch, L., and French, D., *J. Am. Chem. Soc.*, **66**, 130 (1944).
15. Bear, R. S., *J. Am. Chem. Soc.*, **64**, 1388 (1942).
16. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 1707 (1943).
17. Schoch, T. J., *Cereal Chem.*, **18**, 121 (1941). For a complete review see *Advances in Carbohydrate Chem.*, **1**, 247 (1945).
18. Rundle, R. E., and Edwards, F. C., *J. Am. Chem. Soc.*, **65**, 2200 (1943).
19. Rundle, R. E., *J. Am. Chem. Soc.*, **69**, 1769 (1947).
20. Kerr, R. W., and Severson, G. M., *J. Am. Chem. Soc.*, **65**, 193 (1943).
21. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 558 (1943).
22. Senti, F. R., and Witnauer, L. P., *J. Am. Chem. Soc.*, **68**, 2407 (1946).
23. Bear, R. S., and Samsa, E. G., *Ind. Eng. Chem.*, **35**, 721 (1943).
24. Schoch, T. J., and French, D., *Cereal Chem.*, **24**, 231 (1947).
25. Meyer, K. H., Bretano, W., and Bernfeld, P., *Helv. Chim. Acta*, **23**, 845 (1940).
26. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2957 (1942).
27. Tanret, G., *Compt. rend.*, **158**, 1353 (1914); Pacsu, E., and Mullen, J. W., *J. Am. Chem. Soc.*, **63**, 1168 (1941).
28. Hassid, W. Z., and McCready, R. M. *J. Am. Chem. Soc.*, **63**, 2171 (1941).
29. Bear, R. S., and Cori, C. F., *J. Biol. Chem.*, **140**, 111 (1941).

30. Bates, F. L., French, D., and Rundle, R. E., *J. Am. Chem. Soc.,* **65**, 142 (1943).
31. Bourne, E. J., and Peat, S., *J. Chem. Soc.,* **1945**, 877; with Macey, A., *ibid.,* **1945**, 882.
32. Swanson, M. A., and Cori, C. F., *J. Biol. Chem.,* **172**, 815 (1948).
33. Meyer, K. H., and Bernfeld, P., *Helv. Chim. Acta,* **24**, 389 (1941).
34. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.,* **66**, 111 (1944).
35. Rundle, R. E., Foster, J. F., and Baldwin, R. R., *J. Am. Chem. Soc.,* **66**, 2116 (1944).
36. Rundle, R. E., and Baldwin, R. R., *J. Am. Chem. Soc.,* **65**, 554 (1943).
37. Stein, R. S., and Rundle, R. E., *J. Chem. Phys.,* **16**, 195 (1948).
38. Freudenberg, K., Friedrich, K., and Bumann, I., *Ann.,* **494**, 41 (1932).
39. Reeves, R. E., *J. Biol. Chem.,* **154**, 49 (1944).
40. Bridgman, W. B., *J. Am. Chem. Soc.,* **64**, 2349 (1942).
41. Beckmann, C. O., and Landis, Q., *J. Am. Chem. Soc.,* **61**, 1495, 1504 (1939).
42. Coles, J. S., Dissertation, Columbia University, New York (1941).
43. Staudinger, H., *Naturwissenschaften,* **25**, 673 (1937).
44. Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.,* **65**, 618 (1943).
45. Dombrow, B. A., and Beckmann, C. O., *J. Phys. Colloid Chem.,* **51**, 107 (1947).
46. Foster, J. F., and Lepow, I. H., *J. Am. Chem. Soc.,* **70**, 4169 (1948).
47. Senti, F. R., private communication.

CHAPTER VIII

CHEMICAL PROPERTIES OF STARCH

COMPOSITION AND STRUCTURE

1. **Introduction.** Starch is a high polymeric carbohydrate. The repeating unit is glucose and these monomeric units are presumed to be joined by glucoside linkages since substantially 100% theoretical yields of glucose may be obtained by hydrolyzing starch at low concentration in comparatively dilute aqueous solution of acids. Starch shows initially only a very feeble reducing power but as the hydrolysis proceeds, the ability to reduce such reagents as alkaline solutions of copper sulfate, progressively increases.

Minor amounts of non-carbohydrate residues are also found in starch hydrolyzates. These are inorganic ions, fatty acids, other lipids and nitrogenous material, possibly of protein origin. However, with the possible exception of phosphate, these products are now generally believed to arise from impurities which cannot be readily removed when the starch is prepared and purified.

Diastatic enzymes hydolyze starch and the principal end product in this case is maltose. Under favorable conditions some starches yield as high as 70 to 80% of this sugar, which consists of two glucopyranoside units joined by a 1-4, α-glucosidic linkage. Other preliminary data which support the view that this linkage is the principal polymeric bond, that is, that the great majority of the glucose units in starch are joined as in maltose, are as follows. Maltose may be separated from the products arising from an incomplete acid hydrolysis of starch by etherifying or esterifying the reaction products and fractionally distilling off the maltose ether or ester (1, 2). Both Karrer (3) and Haworth (4) and their coworkers observed that when starch products are treated with acetyl bromide this acetolysis reaction yields substantial quantities of maltose derivatives. Finally, Irvine (5) and Haworth (6) and coworkers completely methylated starch, hydrolyzed this ether and found as the principal product, 2, 3, 6-trimethylglucose. Further research disclosed a small amount of 2, 3, 4, 6-tetramethylglocuse and inasmuch as these two methylglucoses were obtained in equal amounts from maltose when similar procedures were used, it logically follows that starch consists of a series, or chain of glucopyranoside units and that these are joined, one to the next, for the most part at least, as they are united in the sugar maltose.

Considerable evidence now exists that chemical bonds other than 1-4, α-glucosidic linkages are also present in starch and the principal problem in starch chemistry in recent years has been the determination of the nature and number of these bonds and the distribution of these anomalous linkages throughout the structure of starch.

The common starches may be fractionated by essentially physical, or physical-chemical procedures into components which differ in respect to both chemical properties and physical behavior. Accordingly, it has become evident that compared to many other carbohydrates, starch is relatively heterogeneous. Therefore structure can best be discussed in relation to the chemical structure of the components, or fractions, which make up the starch granule and composition is given as the relative proportion of these components which differ in structure. This study is additionally ramified because apparently starches from different sources, such as corn, peas or waxy sorghum, differ from each other, particularly in respect to composition.

The view is now generally accepted that the majority of starches contain molecules which can be classified according to one of two quite different structural patterns. One type is a linear polymer and the polymeric bonds are substantially all 1-4, α-glucosidic linkages. The other contains a very large proportion of these linkages also, but periodically throughout the structure, linearity is interrupted by an anomalous linkage, such as, for example, a 1-6, glucosidic linkage which would serve to connect another chain of glucose units to the primary chain of anhydro maltose units and thus create a branched structure. Several structures have been proposed for the non-linear molecules in order to account for the observed chemical and physical characteristics of this fraction.

There is a considerable range of sizes among the molecules of each structural type, even in any one variety of starch. Furthermore, among the non-linear molecules there is some variation in the frequency with which linearity is interrupted and in the length of the terminal branches. Nevertheless, in order to simplify the subject, the custom has prevailed for classifying starch polymer fractions according to whether they are essentially linear or non-linear, and to call the first, amylose, and the second, amylopectin. It should be borne in mind that this classification may be somewhat superficial and arbitrary, since there may very well be molecules which contain so few anomalous linkages that they exhibit the properties of linear polymers and there may be linear polymers in starch so gigantic in size that their linear structure is obscured in many tests, owing to the non-linear configuration they may, or do assume under certain conditions.

The study of the composition and structure of the starches is further complicated by the pronounced tendency of starch molecules, particularly the linear ones, to associate with each other through secondary bonds, such as, for example, hydrogen bonds. Because of the great number of these bonds which form, on occasion, between any two molecules, the total effect of these secondary bonds may be mistaken for the existence of anomalous chemical bonds and the erroneous conclusion may be drawn that a non-linear structure is present. Also, determinations of molecular size and the fractionation of starch into its components are made very difficult because of these intermolecular associations.

Accordingly, in chemical studies, when fractions are designated as amylose or amylopectin, or when subfractions of these primary groups are discussed, it would seem imperative to indicate the procedure by which these starch fractions were prepared and to name the variety of starch used.

2. **Composition.** Several methods have been employed for separating starch into amylose and amylopectin fractions and for subfractionating these two groups further into more homogeneous samples. Schoch (7, 8) has recently presented critical reviews of many of these procedures. For the present, at least, the composition of the starches must be discussed in terms of the results of these procedures. The more important of these involve electrosedimentation of amylopectin fractions which contain phosphoric ester groups, leaching starch granules with hot water and the precipitation of amylose fractions from aqueous sols by the addition of alcohols or other agents which form complexes with this fraction.

Gatin-Gruzewska (9) observed that potato starch pastes could be fractionated by electrophoresis. The amylopectin migrated toward the anode. Samec and Haerdtl (10) and Samec and Mayer (11) used electro-sedimentation, placing the anode at the bottom of the cell to accelerate the sedimentation of a gelatinous potato amylopectin fraction. The results of these many investigations concerning the nature of potato starch fractions have been summarized by Samec (12, 13). Apparently potato amylopectin molecules are esterified with a small amount of phosphoric acid and the compound has sufficient polarity, due to ionization of the phosphate group, to migrate when a potential is placed across the sol. Potato starch was found to contain 73% amylopectin, the balance of 27% being amylose which remained as a limpid solution. The amylose fraction contained little, or no phosphorus and stained blue with iodine.

Hess and Krajnc (14) and Hess and Steurer (15) studied the two potato starch fractions prepared according to the procedures used by Samec and Haerdtl. The fractions were methylated, hydrolyzed, and the percentage of tetramethylglucose determined. By this method of end group analysis, 4.9 to 5.0% of non aldehydic terminals were estimated to be present in the amylopectin fraction, or one terminal for about every 23 other glucose units. The amylose fraction showed one terminal for every 238 glucoses or only 0.47 to 0.50% of tetramethylglucose. Inasmuch as the amylopectin fraction disclosed, by osmotic pressure measurements, the presence of much larger molecules and since many more terminal groups per unit weight were found than for the amylose, a difference in structure was indicated. Hess and coworkers concluded that the structure of potato amylopectin molecules is very much more branched than that of amylose.

Although fractionation by electrophoresis has been applied by many workers to starches other than potato, for example, corn, wheat, and rice starches, the results obtained are questionable since the efficiency of the method depends on one fraction having a much greater, or different polarity than another. This condition does not obtain, in general, with other starches. Few starches contain components which are esterified with sufficient phosphoric acid to develop an appreciable ionic charge in solution. Some may contain impurities in trace amounts which, through adsorption, may confer a small charge on the complex, but in general, this method for the determination of composition must be considered to have limited application and should, for the present, be confined to the study of potato starch.

Leaching of starch with hot water was employed in very early studies. By its use Maquenne and Roux (16) were able to dissolve a portion of starch which could be separated from the swollen granules by centrifuging. The dissolved portion in the centrifugate precipitated spontaneously on standing and could be redissolved in fresh water when heated to a temperature of 150°C. After dissolving and precipitating in this manner several times the recovered material was compared against whole starch. The amylose stained blue with iodine when dissolved. The intensity of the blue color formed by iodine and the whole starch paste was found to be 79% of the intensity of the color formed by iodine and amylose. The dissolved fraction was completely hydrolyzed with malt diastase to give a quantitative yield of maltose, whereas whole starch gave only about 80% of the theoretical yield of this sugar. These two tests, elaborated into more precise systems for measurement, are currently used, among other methods, to differentiate amylose from amylopectin, or linear from non-linear starch polymers.

Tanret (17, 18) applied extraction with hot water to several starches. After three extractions and dehydration of the residue, the following amounts of amylose were found to have dissolved: corn starch, 30%, wheat starch, 27%, and rice starch, 31.5%. Tanret concluded that the amylose which dissolves in increasing amounts under certain conditions consists of a homologus polymer series. With the use of some starches, such as potato, substantial amounts of amylopectin were also found to dissolve in the extraction liquors and in these cases purification was effected by the adsorption of the amylose on cotton.

Sherman and Baker (19) and Baldwin (20) extracted potato starch and found a much lower proportion of phosphorus in the soluble fraction than in the insoluble residue.

Meyer and coworkers (21) studied the extraction of corn starch and found that approximately 6% dissolved in water at 70°C. When this material was recovered by spontaneous precipitation from the extract on standing, it was subfractionated by fractional extraction with water at 90°C. From osmotic pressure and viscosity measurements a minor portion gave calculated DP values between 80 and 160 glucose units and by far the larger portion (88% by weight) was found to have DP values between 160 and 215 (22). A fraction having a DP of 300 was methylated, hydrolyzed, and the proportion of 2, 3, 4, 6-tetramethylglucose in the resulting methylated sugars was determined (23). This value was found to be 0.32%, whereas 3.7% was obtained in methylation experiments on the residual starch after extraction. It was concluded that the extracted amylose contains in each molecule only one terminal glucose unit capable of yielding 2, 3, 4, 6-tetramethylglucose and is, therefore, linear in structure. The amylose was hydrolyzed to maltose by the enzyme β-amylase and was stained blue by iodine. Inasmuch as the extracted residue from starch was found to contain molecules having a DP of 1300, or more, and since methylation studies indicated one terminal end group which gave tetramethylglucose for about every 25 glucose units in its structure, amylopectin was assigned the branched, or ramified structure, which had been deduced by others from studies on whole starch.

Meyer and coworkers also extracted corn starch using progressively higher temperatures and found that over the temperature range of 70 to 90°C. about 30% of the weight of starch could be extracted by many repeated treatments. However, the fractions obtained at higher temperatures, such as 80 and 90°C., showed a materially reduced limit of hydrolysis to maltose with β-amylase.

Kerr and coworkers (24, 25) studied the extraction of corn starch with hot water in order to determine the yield and character of the components obtained under various conditions. Not only were higher temperatures found to give increased yields of extractable solids and amylose, but also the use of defatted starch additionally increased these yields over the temperature range studied. Extraction temperatures in the neighborhood of 75°C. permitted the leaching from defatted corn starch of solids having the highest purity in respect to amylose. About 10 to 15% of the weight of the starch can be extracted with a purity of 85 to 90%. Above this temperature the purity as linear polymer decreases even though the amount of amylose which dissolves becomes larger. Decreasing the ratio of starch from 2.5 to 0.5 g. per 100 ml. of water did not materially increase the yields; neither did repeating the extraction with fresh lots of water on the same sample of starch. When extracts containing amylose are used to extract fresh lots of starch, approximately the same amount of amylose is extracted from the starch as when water alone is used; the dissolved amylose acts neither to solvate other amylose molecules nor to repress its solubility within the granule. That fraction of amylose which will dissolve under a given set of conditions, does so quite readily and its solubility, over the concentration range studied, is determined primarily by its structure and its original physical state within the granule.

Extracts of corn starch were clarified and the amylose crystallized by the addition of butanol to the hot liquors. Fractions obtained at lower extraction temperatures were twice recrystallized from aqueous butanol and formed rectangular platelets, as shown in Fig. 58. These were found to be soluble in hot water, gave an X-ray diffraction pattern as shown in Fig. 59, and were hydrolyzed in dilute solution by β-amylase to an extent corresponding to about 95% of theory for an unbranched chain of anhydro maltose units. At this point in the hydrolysis the reaction rate was materially reduced.

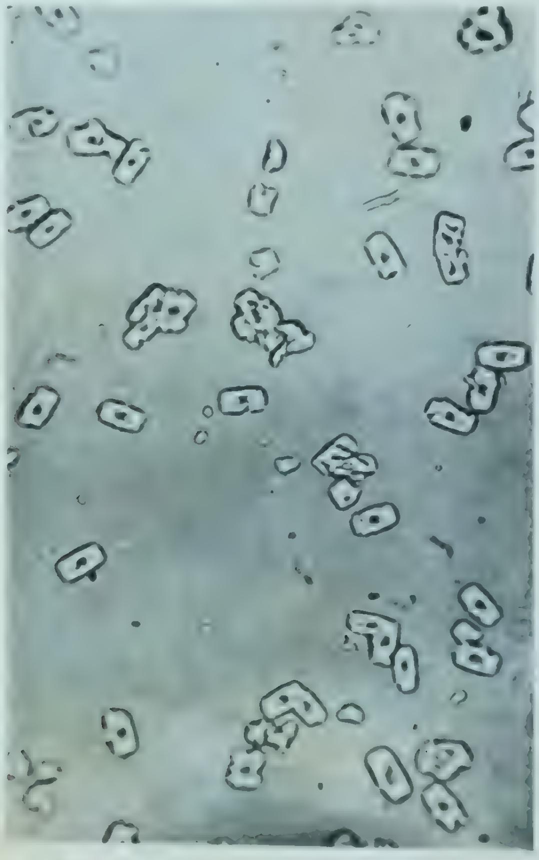


FIG. 58. Corn, crystalline amylose.

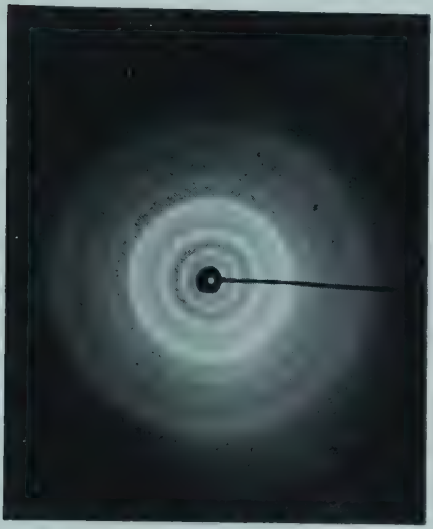


FIG. 59. X-ray diffraction pattern of corn, crystalline amylose.

apparently due to retrogradation effects. The crystals were found to have a very high blue color value when solutions were treated with iodine and examined spectrophotometrically (26) and to combine with 0.20 to 0.21 g. of iodine per gram of amylose when titrated, potentiometrically with this reagent (27). Bates *et al.* (28) examined the purity of the crystals by potentiometric titration with iodine, a method originated by these workers, and Rundle and French (29) studied the crystal structure of the amylose and the amylose-iodine complex. The platelets were found to consist of linear molecules arranged in the shape of helical coils with the axes of the helices perpendicular to the largest face of the crystal.

Crystalline amyloses were prepared from potato and tapioca starches in a manner analogous to that employed with corn starch. However, these amyloses crystallized as needle-like shapes which tended to form star-like clusters (Fig. 60). These also combined with 20%, or more, of iodine, had very high limits of hydrolysis to maltose with β-amylase, but had significantly higher solution viscosities than the corn amylose. Yields were comparable to that for corn starch, between 5 to 10%.

Other amylose fractions, in crystalline form, were prepared from corn starch by re-extracting at higher temperatures the starchy residues from previous extractions at lower temperatures. Fractions solubilized at 90°C. and higher, and crystallized from aqueous butanol, resembled those obtained at lower temperatures from potato and tapioca starches in respect to shape of crystals, higher solution viscosity, better stability in water solution and lower alkali lability value. Fractions solubilized at 90°C., and insoluble at this temperature had intrinsic viscosities in ethylenediamine which were very

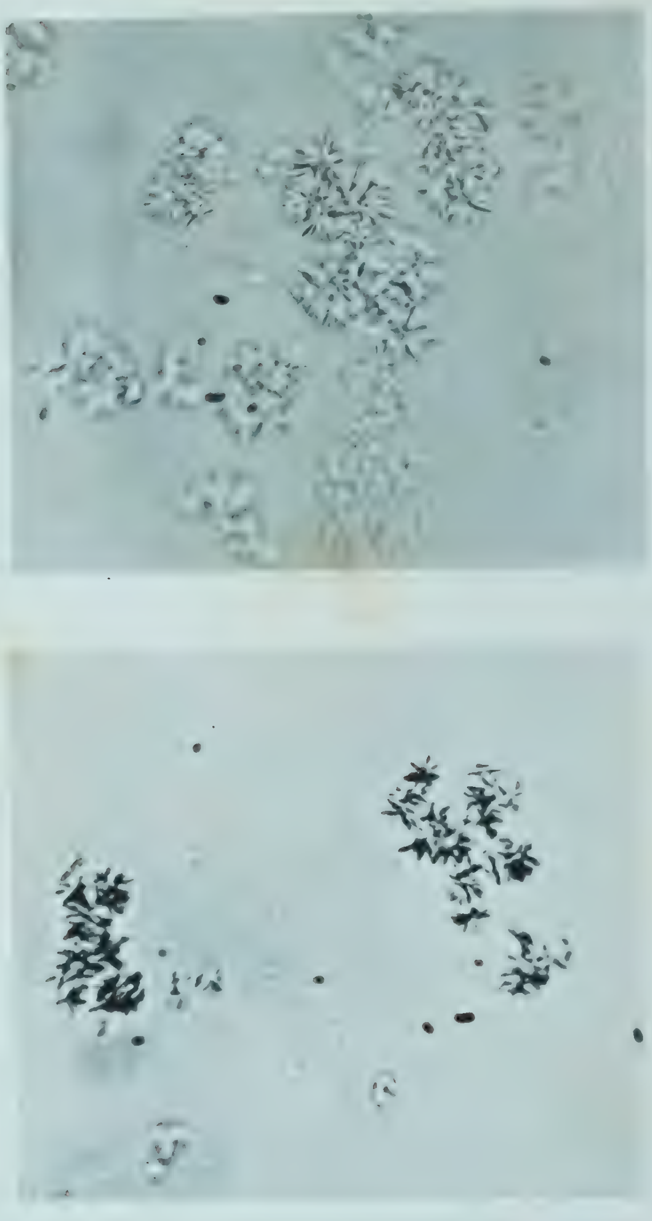


FIG. 60. Potato and tapioca crystalline amyloses.

nearly double the value of 0.66, which was found for the fraction soluble at 75° C. The fractions having higher viscosity showed less tendency to retrograde, or spontaneously precipitate from solution and had alkali lability values as low as 23, considerably lower than the value of 35, found for the fraction soluble at 75° C. The alkali lability value, when determined according to arbitrarily defined conditions, such as by the method of Schoch and Jensen (30) is at least a qualitative index of molecular size, particularly for linear starch polymers; the higher the value, the shorter the chain

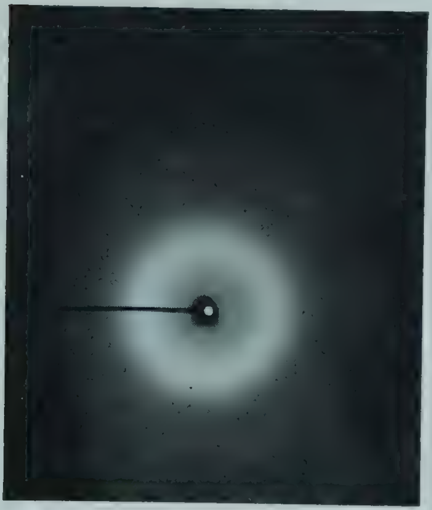


FIG. 61. X-ray diffraction pattern of a corn amylopectin subfraction.

length. The iodine color value of all of these fractions was found to be of a high order. Later work (31) on osmotic pressure measurements of the acetates of these corn starch fractions in chloroform has shown that the fraction soluble in water at 75°C. has an apparent DP_n of 225 and the fraction soluble above 90°C., 575.

Inasmuch as about 20% of corn starch, when solubilized, can be crystallized with butanol, a logical interpretation of the above results is that this proportion of the starch, at least, is composed of chain-like molecules which are made up of anhydro glucopyranoside units and that these units are substantially all joined, one to the next by 1-4 α-glucoside linkages, as in maltose. Chain lengths vary over a wide range, at least between the limits DP 200 to 600. Tapioca and potato starches do not contain linear chains, in substantial amounts, as short as those found in corn starch.

The greater colloidal stability of the fractions of higher molecular weight could be explained on the basis of altered configuration, such as the very long molecules assuming a folded, fluted, or ball-of-twine shape in solution and this would make more difficult association between these molecules at a sufficient number of points so as to create an insoluble particle exceeding colloidal dimensions. For it is a fact that freshly prepared solutions of these amyloses in water, as they approach infinite dilution, exhibit about half the specific viscosity shown in better solvents such as ethylenediamine and normal solutions of potassium hydroxide. This could be taken to indicate the formation of a much more compact molecular shape in neutral aqueous solution.

An alternate theory to explain the greater colloidal stability of the larger amylose molecules is that they are branched to some extent. This proposal

TABLE XII

Some Properties of Corn Starch Fractions and Sub-Fractions

			Intrinsic viscosity					Iodine	
Fraction	Fractionation	DP_n	Ethylene-diamine, 25°C.	Water 25°C.	1N KOH 35°C.	Ferricyan-reducing number	Alkali labile No.	Affinity, % I	Molecular extinction coef.
Amylose	Pentasol precipitated	455	1.258		1.20	1.43	22	19.2	3730
Amylose	Butanol precipitated	435	1.065	0.525	1.10	1.66			3500
Amylose									
Subfraction I	Ethylenediamine, ethyl ether subfractionation	250	0.593	0.245	0.54	2.88	31.2	20.2	3830
Subfraction II		390	0.775		0.65	2.12	26.7	19.6	3830
Subfraction III		525	1.130	0.560	0.93	1.46	23.2	20.4	4210
Subfraction IV		675	1.350		1.10	1.02	19.8	21.0	4430
Amylose									
Soluble 75°C	Hot water and butanol crys.	225	0.655		0.58		35	20.0	3630
Soluble 90°C.		575	1.172				24	19.5	
Insoluble 90°C.	NaOH, butanol	545	1.158				23	18.8	
Amylopectin	Butanol non-precipitated	1450			1.25	0.46	4.9		
Amylopectin									
Subfraction I	Butanol and Methanol	1800	1.445		1.45	0.38	3.2	2.4	
Subfraction II		1200			1.06	0.54	5.6	1.5	
Subfraction III		450	0.526		0.54		13.7	0.0	

meets some of the objections to the first which is not entirely satisfying. In the first place, linear molecules of DP 200 are relatively very long compared to their width and it would seem that they could be just as kinked, or fluted, or ensnarled as molecules several hundred units longer. If the comparison were being made between molecules with a DP of 10 to 20 against molecules having a DP of several hundred, naturally, a marked difference in colloidal behavior would be expected. Secondly, when very dilute solutions of the amylose fractions are prepared in $1N$ KOH, neutralized, and viscosity determinations immediately made, fractions of DP 200 to 250 also show a loss of 50% in intrinsic viscosity on neutralization, just as do fractions of DP 600. Thirdly, pronounced kinking, folding, or forming a ball-of-twine shape, should bring units of the same molecule into closer proximity and should favor intramolecular associations of the same type as those which form between linear starch molecules when sufficiently close together. It is well known that these associative bonds very greatly impede the action of agents which form complexes, for example, iodine, higher alcohols, and enzymes, because retrograded, or retrograding solutions of amylose react feebly, if at all, with these complexing agents. Therefore, if a marked difference in configuration applies only to the larger amylose molecules, one would expect these agents to be much less active with them than when introduced into solutions of the shorter chains. Such, however, is not always the case. For example, iodine reacts readily with starch fractions of DP 600 to form a complex which contains an amount of bound iodine per unit weight and a blue color value of the same order of magnitude as the complex formed from linear starch chains of DP 225.

Therefore, until more precise analytical methods are developed which permit a more positive interpretation of the results on starch composition studies, one may assume that the data obtained on fractions prepared by extraction show that some starches contain a small percentage of unbranched molecules, possibly 10% in the case of corn starch, and that the balance is composed of molecules which vary both in respect to frequency of branching and average length of terminal branches. The common starches contain about 20%, by weight, of components which exhibit the properties of linear polymers and there is a wide distribution of molecular sizes in both the linear and non-linear polymer fractions.

Some varieties of starch, of course, have compositions which are quite different. At opposite extremities are the waxy starches which contain less than 1% of hot water extractable and butanol precipitable amylose and starches, such as that from certain peas, which contain as high as 50 to 75% of linear polymers and which will be discussed in the following section.

The remarkable ability of linear starch polymers to form insoluble complexes with many agents, some of these complexes being highly crystalline in structure, was brought to attention by the work of Schoch (32, 33), who first used butanol to fractionate autoclaved starch sols into two fractions. The fraction rendered insoluble was found to be relatively less stable in colloidal solution and had a higher alkali lability value than the fraction which was not precipitated and Schoch concluded that a difference between the two fractions was indicated in respect to structure. Butanol was found to precipitate 22% of the weight of both corn and potato starches but nothing from waxy maize.

Earlier investigators had observed the formation of crystal-like material when complexing agents such as alcohol and pyridine were added to starch solutions, but apparently had failed to recognize that the insolubilized material was different chemically from the starch substance which does not readily crystallize. Weigel (34) used hot aqueous ethanol to fractionate starch and obtained a 10% yield of crystalline material which he recognized as being an amylose. However, Meyer and Fuld (35) found that this product was not completely converted to maltose by the enzyme β-amylase and concluded that the ethanol precipitate contained amylopectin as well as amylose. Later, Weigel (36) recrystallized his amylose samples from aqueous butanol and very likely obtained some purification. He also showed that amylose fractions formed complexes in aqueous solutions of isopropyl, isobutyl, and isoamyl alcohols, dioxane and ethylene chloride.

Bear (37) showed that amylose forms complexes with normal and branched chain aliphatic alcohols, the lower ketones and fatty acids and aromatic derivatives such as phenol, benzyl alcohol, aniline and benzaldehyde. Whistler and Hilbert (38) concluded that amylose forms a complex with any agent which possesses either donor or acceptor groups for hydrogen bonding and extended the list to include esters, mercaptans, pyridine and nitroparaffins. The yield of amyloses obtain from several starches by these workers, using the lower nitroparaffins as fractionating agents, is given in Table XIII. The results of other investigators, using various precipitating agents, are also given in this table.

Haworth *et al.* (39) have used cyclohexanol, menthol, borenol and thymol to fractionate slightly alkaline potato starch sols. These investigators reported that a combination of thymol and cyclohexanol leaves an amylopectin fraction which gives a much lower iodine coloration value than when either butanol or thymol is used as a fractionating agent. Haworth and coworkers are led to believe that more than one amylose type is present, in potato starch at least, and that conditions which favor the precipitation of one may

TABLE XIII

Composition of Starches

Per cent Amylose, or A-Fraction

	Gravimetric Methods							Iodometric Methods				
Starch	Butanol (Schoch)	Pentasol (Schoch)	Oleic Acid (Schoch)	Butanol (Higgin-botham)	Nitro-ethane (Whistler and Hilbert)	1-Nitro-propane (Whistler and Hilbert)	2-Nitro-propane (Whistler and Hilbert)	Thymol (Haworth, *et al.*)	Pen-tasol (Schoch)	Butanol (Higgin-botham)	Butanol (Bates *et al.*)	Hot Water-butanol (Kerr)
Corn	22	28-29	29	32	22-23	26-28	26-29		28	29.5	21	29
Tapioca	21			20		20-21			17	17.5	17	20
Potato	22			—		26-27		20-22	20-21	——	22	25.5
Wheat	—			—		31-32			26	——	24	
Sago	—			28					—	27	27	
Rice											17	
Banana											20.5	
Popcorn											23	
*Peas	60										—	
Easter lily		25									34	
Waxy maize	0	0									0	0
Waxy sorghum	0	0									0	
Waxy rice											0	
Waxy barley											0	

*Certain wrinkled varieties, (Hilbert and MacMasters) not necessarily precipitate others. They found that the high level of purity obtained by repeatedly reprecipitating an amylose fraction with one complexing agent is not necessarily attained with fractions similarly prepared and treated with other complexing agents.

In later work Schoch (7) has stated a preference for the use of Pentasol* as a fractionating agent. The yields of precipitable fractions are higher and the iodine affinity of the fraction not precipitated is lower. However, the iodine affinity of the precipitates from various starches may also be lower. When the Pentasol precipitable fraction of corn starch was recrystallized several times from aqueous butanol, Schoch (7) reported that the iodine absorbed by the products increased progressively from 0.165 to 0.190 g. of iodine per gram of starch fraction. Moreover, it will be noted (40) that during recrystallization with butanol the larger yield of corn starch fraction obtained with Pentasol (about 28 to 30%) decreases to about 20 to 22%, the same value which is obtained when only butanol is used as the precipitating and recrystallizing agent.

Schoch has concluded (8) that subfractionation may occur during recrystallization.

Schoch has proposed (8) that the fraction of starch which is precipitated by higher alcohols be called the A-fraction. The term amylose is considered to be too indefinite. This is undoubtedly true as was indicated earlier in this discussion. However, in this manner of expressing composition as A- and B-fractions, it would seem necessary to denote the starch used, name the alcohol employed, and state the conditions under which the fractionation was made and one of the major objections to classifying components as amylose or amylopectin would not have been surmounted. For it is apparent that the amount and character of the precipitate obtained from starch varies within limits with alcohol used, the temperature at which the starch and alcohol mixture is held for precipitation, and the time allowed for the precipitation to take place. Tertiary butyl alcohol is so soluble in water that substantially all starch solids may be precipitated from aqueous solution by the addition of this alcohol. Tertiary amyl alcohol is soluble in water only to about the same extent as butanol, but in contrast, this alcohol precipitates nearly all of corn amylopectin from 3% aqueous solutions of the latter, as a gel like mass when mixtures are held at or below 20°C. Butanol will precipitate substantially more than 22% of corn starch if the primary centrifugate is held under aseptic conditions for several months time at lower temperatures. And furthermore, present data would seem to indicate that the fraction precipitable by Pentasol is not the same as the fraction precipitable by butanol but represents a cut farther over in to the series of starch components formerly classified as amylopectin.

---

*Pentasol is the trade mark for a commercial mixture of various primary amyl alcohols, marketed by Sharples Solvent Corporation.

Kerr and coworkers (41, 42) have studied the subfractions of that part of autoclaved corn starch sols precipitated by butanol. These were prepared by dissolving the fraction in ethylenediamine at 25°C. and adding sufficient ethyl ether to form two liquid phases. The solvents were used in such proportions that the subfractions which dissolved in the upper layer after each addition of ether would have approximately equal weights. The subfractions were recovered from each of the upper layers by the addition of excess ether, washing the precipitates with several portions of ether and recrystallizing the residues from neutral aqueous solution by the addition of butanol. Molecular weights were determined (42) by measuring the osmotic pressures of the acetates in chloroform. Apparent DP_n values at infinite dilution were found to vary over the range of 250 to 675. The parent amylose fraction had a DP_n value of 435. Another linear corn starch fraction prepared by precipitation with Pentasol and recrystallization with butanol had a DP_n value of 455 (40), whereas a second Pentasol precipitated corn starch fraction gave a value of 495.

The subfraction of the butanol precipitated corn amylose first to dissolve in the upper layer on the addition of ether had the lowest DP_n value and always crystallized from aqueous butanol as thin, well-shaped rectangular platelets. They were in all respects quite similar to the fraction solubilized from corn starch by water at 70°C., and described previously. The subfraction which remained finally in the lower layer had the highest DP_n value and crystallized as needles. With increasing DP_n values, the subfractions showed increasing intrinsic viscosities, both in ethylenediamine and KOH solution, possibly increasing iodine binding power, and decreasing reducing value, using alkaline ferricyanide reagent. These results are summarized in Table XII, together with the properties of other subfractions. Earlier views that approximately 20% of corn starch consists of linear polymers with a fairly uniform distribution in molecular sizes between DP 200 and 600 appears to be fairly well confirmed.

Data from other subfractionation studies on corn starch fractions indicate that beyond the limit of DP 600, another 10 to 20%, or more, consists of polymers which show several of the characteristics of linear starch molecules. Why they exhibit some, but not all of the properties shown by the shorter linear chains is not known at present. Kerr (43) subfractionated that part of corn starch which is not precipitated by butanol. A combination of butanol and methanol was added in progressively larger amounts to aqueous solutions of the starch fraction. The first subfraction which precipitated stained a greyish blue with iodine, had a very low alkali labile number of 3, from which a DP_n of the order of 2000 is estimated. The preparation was semicrystalline in nature and had an intrinsic viscosity in ethylenediamine of 1.45, which is higher than that of any of the corn amylose subfractions studied. It was concluded that these molecules were more extended in shape than the amyloses.

The amylopectin subfractions which precipitated at higher alcohol concentrations redissolved readily in water and formed sparkling clear, very stable solutions. They had alkali lability values as high as 13.7, indicative of a molecular size below average for corn starch, gave no blue color with iodine and showed a relatively low intrinsic viscosity in ethylenediamine. An X-ray diffraction pattern was obtained characteristic of substantially amorphous substances. (Fig. 61). The osmotic pressure of the acetate of the amylopectin subfraction of lowest molecular weight (III) was measured in chloroform and a DP_n of 450 in this solvent was calculated, as is shown in Table XII. Acetates of corn amylopectin subfractions I and II, prepared in pyridine by the Pacsu method were insoluble in chloroform. However, acetates of these subfractions prepared by dispersion of the starch substance in formamide, followed by acetylation with the anhydride and using pyridine as catalyst, are chloroform soluble and osmotic pressure measurements (31) gave DP_n values of the order of 1800 for subfraction I and 1200 for subfraction II.

Recently, Meyer and coworkers (44) have fractionated corn starch by precipitation with butanol, according to the method of Schoch, and have examined the fractions by an end group assay method, using dinitrosalicylic acid to form a brown colored product with the terminal aldehyde groups. The analytical procedure was standardized in a photometer, by using as standards glucose, maltose and a short chain amylose, the molecular weight of which had been determined by osmotic pressure measurements. A DP of 450 was found for the corn amylose prepared by Schoch's method (A-fraction), while amylopectin fractions were obtained with DP values of 270 and 280. Meyer and coworkers also applied Schoch's method of fractionation to corn starch which had first been extracted with water at 70°C. and obtained an *amylose fraction superieur* (A_2-fraction) with a DP of 2100, whereas the extracted amylose, after precipitation with butanol (A_1 fraction) had a DP of 250. Thus Meyer and coworkers are in very good agreement with the results of Kerr and coworkers (40, 42) as to the molecular weights of corn crystalline amylose (amylose A_1 according to Meyer), of the total corn amylose fraction (A-fraction according to Schoch) and in fair agreement on the lowest molecular weight amylopectins from corn starch. However, Meyer finds a considerably greater DP for *amylose fraction superieur* than did Kerr for any corn amylose subfractions which were obtained either by butanol precipitation of corn starch after it had been extracted with hot water and dissolved in NaOH, or obtained by subfractionation of whole corn amylose, using ethylenediamine and ethyl ether in a solvent phase separation technique, and it appears to approach more closely, or to include, in part at least, the first subfraction of amylopectin described by Kerr.*

Recently, methods have been developed for estimating the composition of starches, which combine both an isolation of amylose fractions and a comparison of iodine complex formation by the standard amylose fraction and the parent starch, either by potentiometric titration or by spectrophotometric measurements. Application of the method, in principle, was attempted in early work by Maquenne and Roux (16), but appears to have been neglected until the extensive researches of Rundle, French, and coworkers on the nature of the amylose-iodine complex, pointed out the importance of the reaction involved. Bates *et al.* (28) determined the amount of iodine bound by amylose fractions by means of potentiometric titration with iodine. In this preliminary work, the crystalline amylose isolated from corn starch according to the method of Kerr and Severson (24) was used as a standard since, of the various amyloses tested, it showed the highest percentage of bound iodine. Using this standard, several starches were analyzed for amylose content and these results are shown in Table XIII. Schoch (8) has expanded on this method by repeatedly recrystallizing the A-fraction from each starch, with butanol, determining the amount of iodine bound by each A-fraction and dividing this value into the amount of iodine bound by the starch. For example, Schoch found that the A-fraction from corn starch combines with 19% iodine. Purified corn starch combines with 5.3%. Therefore, dividing 5.3 by 19, one obtains 28% as the proportion of A-fraction in the starch. In a similar manner Schoch has estimated the proportion of A-fraction in other starches and these values are also given in Table XIII.

Hassid and McCready (45) separated potato amylose by a method involving electro-sedimentation, treated the amylose solution with iodine and determined the fractional transmission of light through the blue solution,

---

*In applying Schoch's fractionation procedures to residual starch which has been leached with hot water, it would seem quite possible that the autoclaving step was not sufficient to dissociate the hot water treated starch and that the *amylose superieur,* or A_2-fraction obtained from this residue by the addition of butanol may have been associated with amylopectin molecules, or possibly highly associated with itself. In analogous procedures used in earlier work by the author, for preparing the amylose subfraction not solubilized at 90°C., the hot water extracted starch residue was first dissolved in sodium hydroxide.

using a light filter appropriate to the blue color. In a similar manner, fractional transmission values were determined for known mixtures of the amylose and amylopectin from which a standard curve was made. The fractional transmission for the blue color of whole potato starch was measured and from the position of this value on the standard curve, an amylose content of 20% was found for potato starch. Kerr and Trubell (26) developed a similar spectrophotometric procedure, using as standards a crystalline amylose and an amylopectin subfraction from the starch under study. The amyloses were prepared by hot water extraction and butanol crystallization. The amylopectins were subfractionated by the butanol-methanol technique referred to above for corn starch and the amylopectin subfractions last to precipitate and which gave no blue color whatsover with iodine, were used as standards. Since all the amyloses tested showed a minimal light transmission value in the range of wavelengths corresponding to 600-620 mμ when they were stained with iodine, all analytical measurements were made with a light source of 610 mμ. The amylose content of three starches were found to be as follows: corn starch (defatted), 29%, potato starch, 25.5%, and tapioca starch, 20.0%. Baldwin *et al.* (46) also developed a spectrophotometric procedure. These investigators titrated amylose solutions with iodine and used the spectrophotometer to determine, by light transmission measurements, the end point in the formation of the complex. From the percentage of iodine bound by the amylose, and comparing this value with that obtained for the starch, one may calculate the amylose content of the starch. The amylose and amylopectin fractions were prepared by the butanol precipitation method of Schoch, with the added procedure of treating the amylopectin fractions with cotton to remove the last traces of amylose impurities.

However, Baldwin *et al.* found that their purified amylopectin fractions, with iodine, gave an absorption spectrum and that the wavelength of maximum absorption became progressively larger for the series, waxy maize, potato, and corn. The absorption was thought to be due to unbranched sections of structure in the amylopectins and the higher maxima were thought to indicate a less branched structure. Furthermore, for the amyloses, both the molecular extinction coefficient and the wavelength of maximum absorption for the complex increased with the average chain length in the sample.

It now seems to be fairly well established that the amount of iodine bound in complex formation varies, within limits, with the chain length of the amylose. Horan (47) studied a series of amyloses which were prepared from acid hydrolyzed potato starch samples and which showed a decreasing rder of chain length. The amount of iodine bound by these amyloses decreased from about 19 to 9%.

Therefore, since amylose fractions and subfractions vary somewhat in espect to their iodine value and since some amylopectin fractions, or subractions, may contribute to the effect used to estimate the amylose content of a starch, the results of iodometric analyses should be considered in reference to the standards used in the analysis. Even so, the results do not necessarily indicate that the starch under study actually contains the amount of standard found by analysis. For example, Schoch (7) found 21% A-fraction in tapioca starch by butanol precipitation but only 17% by potentiometric titration with iodine. An even larger discrepancy was found for Easter lily bulb starch. Higginbotham and Morrison (48) also found about the same difference in results for tapioca starch, when analyzed by gravimetric and iodometric methods. These workers concluded that the differences between amyloses are not entirely due to differences in chain lengths. The more colloidally stable, such as tapioca, may contain some, slightly branched molecules, which view is in harmony with that presented above. Iodometric analyses, and other methods which express starch composition in terms of two components, are of considerable value in characterizing starches and starch products. However, the two component theory is, possibly, an over simplification of actual starch composition and until serious structural studies have been made on fractions and subfractions which have been made available for study in recent years, then, as suggested by Kerr and Trubell in presenting their spectrophotometric method, the results should be taken with some reserve.

3. **Structure.** The chemical structure of high polymers is deduced from a variety of both chemical and physical measurements. Unfortunately, the methods which are available for carbohydrates of high molecular weight such as starch are such that the results of no one test positively confirm one theory of structure to the exclusion of all others. However, these tests are many and are, in general, sufficiently independent so that the results, taken as a whole, overwhelmingly favor the view that the common starches are composed of at least two different types of molecular structures. The two extremities in types are, as indicated above, a linear, unbranched chain of glucopyranoside units, joined one to the next by 1-4, α-glucoside linkages. The other is a highly branched, laminated or ramified structure wherein not only are the majority of glucopyranoside groups united as in the first structure, but also, at frequent intervals throughout the structure, a glucopyranoside unit is joined at carbon number six through a glucoside linkage to a third unit, thus creating a branch.

The more important tests or procedures used to deduce starch structure are, exhaustive methylation, hydrolysis by enzymes and by acids, the iodine reaction, oxidation, other aldehydic end group reactions, viscosity, osmotic pressure, sedimentation and diffusion, X-ray diffraction and other physical measurements the results of which indicate size and shape.

Exhaustive methylation is one of the more important tools of organic chemistry for determining the structure of starch. By its use, one may deduce which carbon atoms are involved in ring structure within the glucose molecules from which the polymer is built, the positions at which the glucoside linkages join these monomer units together, the distribution between these types of glucoside linkages and, in conjunction with molecular weight determinations, whether the polymer is linear or branched and if branched, the approximate number of terminal branches. Most of the work in this field, however, was done on whole starch. The extension of this work to the more homogeneous fractions which are now available has proceeded slowly due, no doubt, to certain limitations of these procedures and a decision first to overcome these limitations so as to permit a more precise interpretation of results.

Exhaustive methylation involves treating the starch, or fraction, in basic media, such as, for example, sodium hydroxide or liquid ammonia and sodium, with methyl sulfate or methyl iodide. This treatment is repeated many times so that finally each hydroxyl group present in the original carbohydrate is derivatized into a methyl ether group. The methylated starch is then split with acid into methylated sugars. The various groups of end products such as the di-, tri-, and tetramethylglucoses, or their glucosides, are then separated, one from the other by some preferred procedure. Fractional distillation is commonly used. Inasmuch as methyl ether groups are fairly stable to acid, the methylated glucoses should contain all of the substitutions which were made in the starch sample. Therefore, any free hydroxyl groups found in the methylated glucoses may be assumed to be a position where either a glucoside linkage existed with another glucose unit or where the oxygen ring within the sugar molecule is attached.

Irvine (5) applied this procedure to starch and found that very nearly all of the methylated sugar obtained was 2, 3, 6-trimethyl glucose. Therefore, since carbons 4 and 5 were not methylated nearly all of the glucose units in starch either are joined together by glucoside linkages from carbons 1 to 5 and the sugar units contain furanyl ring structures, or the glucoside linkages are between carbons 1 and 4 and the glucose units are pyranoses. Haworth Hirst and their coworkers studied glucose, maltose and starch. Haworth showed that glucose exists predominantly in the pyranose form and he obtained equal amounts of 2, 3, 6-trimethylglucose and 2, 3, 4, 6-tetramethylglucose from maltose. The tetramethyl derivative shows that this "terminal" or end group in maltose contains an oxygen ring involving carbon number 5, and the trimethylglucose that this sugar unit not only is a pyranose but also was joined at carbon 4 to the other sugar unit by a glucoside linkage. Accordingly, Haworth assigned the structure shown in Fig. 62 to maltose (49). Methylation of starch gave 2, 3, 6-trimethylglucose as the principal product and in addition, 4.5% of 2, 3, 4, 6-tetramethylglucose. From these results it follows that the glucose units in starch are pyranoses, that they are joined together as in maltose (for the most part, at least) and that for about every 20 to 25 glucopyranose units (designated as B, Fig. 63) which yield 2, 3, 6-trimethylglucose, there is one terminal glucopyranose unit (designated as A) which yields 2, 3, 4, 6-tetramethyl glucose. Several starches such as potato, corn, waxy maize, and canna were found to give approximately the same results from methylation experiments (6, 49-54).

Estimations of the molecular weight of starch molecules from their reducing power (55), viscosity and osmotic pressure (56, 57, 58) indicated an average molecular weight corresponding to a degree of polymerization (DP) of many hundred glucose units. Therefore it became necessary to determine whether the shorter unit lengths, which were indicated from methylation experiments, are joined together by chemical bonds or by physical forces, and, if chemical bonds, how they differed from the normal glucosidic linkages. Further methylation studies on glycogen (59, 60),

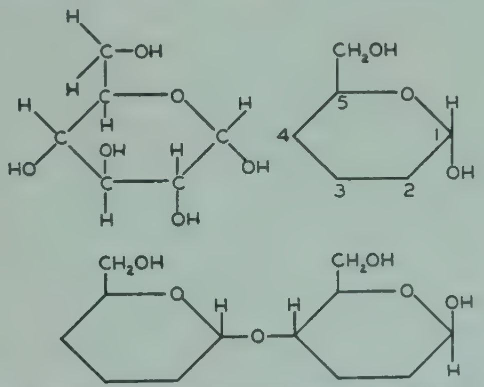


FIG. 62. Upper row: structure of glucose, simplified form of notation given at right; lower row: structure of maltose.

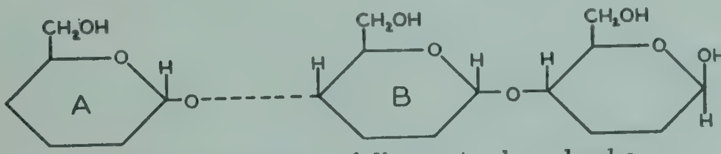


FIG. 63. Structure of linear starch molecules.

showed a yield of tetramethylglucose which indicated a unit chain of some 12 to 18 glucose residues. In addition, a yield of dimethylglucose, about equal to the yield of tetramethylglucose was also obtained. Oxidation of the former with nitric acid to methyl *d*-dimethoxysuccinate indicated that it was mainly, 2, 3,-dimethylglucose. Inasmuch as a DP of several thousand was found by Carter and Record (61) for glycogen, Haworth *et al.* (60) proposed that the shorter units were joined together to form a large, laminated molecule, as shown in Fig. 64, through 1-6 glucosidic linkages. The amount

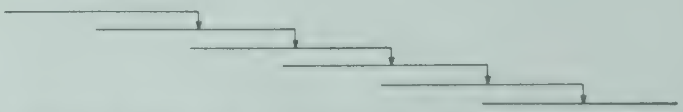


FIG. 64. Laminated structure for branched molecules (Haworth *et al.*, 1937).

of 2, 3-dimethylglucose found would indicate that for approximately every terminal group which gave tetramethylglucose there was another glucose unit, which, in addition to the two normal linkages connecting it in the chain, was connected to a third glucose unit at carbon 6. These workers suggested that this proposed structure applied, possibly, to starch as well as glycogen.

Almost simultaneously, Staudinger (56, 57, 58) proposed a highly branched structure for starch, as shown in Fig. 65 to account for the following facts: that methylation experiments indicate one terminal for about every 25 other

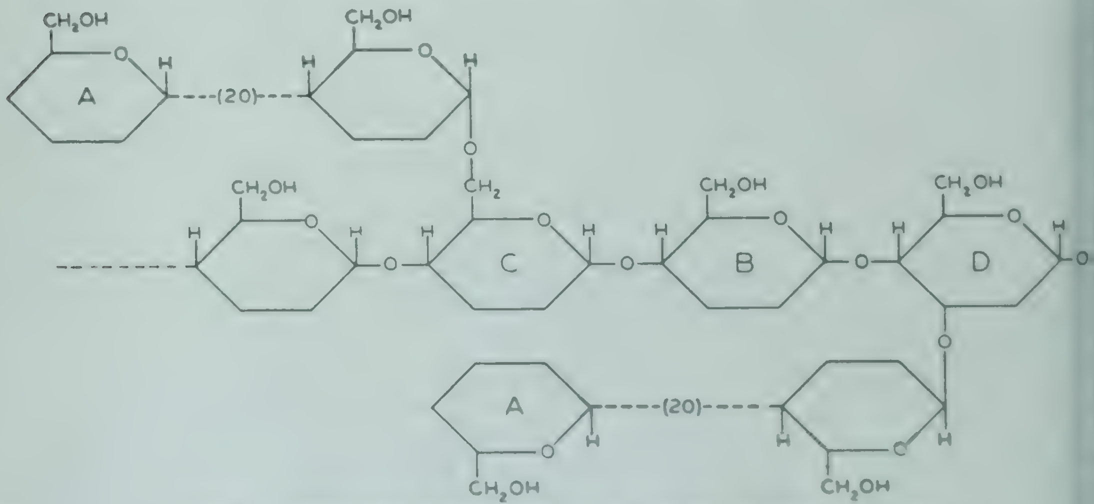


FIG. 65. Structure of branched starch molecules (Staudinger, 1937).

glucose units; that starch shows an average DP of 1000, or more, from osmotic pressure measurements; that this DP is unchanged after derivatization into the acetate, and from the acetate into the methyl ether; that its specific rotation is consistent with this value for DP; that the specific tation is substantially unaltered after acetylation and saponification of he acetate; and that the viscosity of starch is substantially less than that of he linear polymer, cellulose. Acid degraded cellulose, which gave osmotic ressures of the same order as that of starch were used for comparison.

Freudenberg and coworkers (62-65) completely methylated starch in liquid mmonia, using methyl iodide and sodium. Both 2, 3- and 2, 6-dimethyl-lucose were obtained as end products, which results would be anticipated rom the structure proposed by Staudinger. However, Freudenberg showed hat 2, 3, 6-trimethylglucose, the principal product, is susceptible to ydrolysis and that the first reaction product is 2, 6-dimethyglucose. There-ore the presence of this dimethyl sugar in the hydrolyzate from methylated tarch was not thought to be significant and it was concluded that branch-ng in starch is limited to 1-6, glucosidic linkages. Even these were thought o be not as numerous as previous data, based on the percentage yield of dimethylglucose, had indicated. Hirst and Young (66), using improved tech-iques, also found dimethyglucose. The results of Barker *et al.* (67) indi-ated that branching in starch molecules is through 1-6, glucosidic linkages. These results led Haworth (68, 69) to believe that the laminated structure, roposed from earlier studies on glycogen, was confirmed for starch.

The work of Meyer, Wertheim and Bernfeld (23) on corn starch frac-ions and of Hess and Krajnc (14) on potato starch fractions, has already been mentioned. Both groups of investigators showed from tetramethyl-glucose determinations and relative molecular weight measurements that the amylopectin fraction is highly branched, whereas amylose contains very few, f any, branches. Meyer concluded that the amylose fraction prepared by eaching corn starch with hot water is unbranched. Hassid and McCready (70) prepared potato amylose by extracting the starch with hot water and from a methylation, end group analysis, and viscometrically determined mole-cular weight, concluded that this fraction was unbranched, also.

Little work has been published on the methylation of fractions obtained by more recently developed procedures, such as butanol precipitation, or on subfractions obtained from these products. However several papers have appeared on improvements of some phase of the exhaustive methylation technique.

The first obstacle encountered in the use of methylation procedures is the reluctance of starch to derivatize completely. If all the hydroxyl groups in the starch structure are not converted to methoxy groups before the hy-drolysis step, then the validity of deductions based on the distribution between di-, tri- and tetramethylglucoses found in the hydrolyzate is materially weakened. A common methylation procedure is to treat the starch, or fraction, in 5% sodium hydroxide solution at 50°C. with methyl sulphate and 30% sodium hydroxide. Usually, 10 to 15 repetitions of the treatment are required. Starch is susceptible to degradation in alkaline, aqueous solutions and it is also possible that during this prolonged treatment atmospheric oxidation may be initiated at points along the structure, such as the many alcoholic hydroxyl groups. One is not sure, therefore that the final methylated product is representative of the original starch sample.

In order to facilitate the methylation reaction, Freudenberg treated starch with methyl iodide in liquid ammonia. Sodium was added progressively with methylating reagent. However, in using the Freudenberg procedures, one notes a considerable decrease in the viscosity of the reaction mixture when the methoxyl content approaches 45%. Meyer, and others, have criticized this method in that a reduction in viscosity may indicate a reduction in molecular weight. If this reduction is brought about by scission at the normal glucoside linkages, then new end groups may be formed during the methylation procedure that were not originally present. However, scissions at other points, or by various mechanisms are apparently possible in alkaline media, and the analytical results from methylation, may or may not be materially altered. Hirst and Young (71) methylated rice starch under various conditions and obtained products the viscosity of which varied over a broad range. Nevertheless, the end-group analysis was the same in all cases. Even after the methylated starch had been treated further with oxalic acid and the molecular weight had fallen to about 20,000 re-methylation gave the same analytical result for tetramethyl methylglucoside. Hess and Lung (72, 73) also found no correlation between viscosity and analytical results. These workers did find however, that when potato starch was methylated in an atmosphere of hydrogen and the methylation completed by their anisole method, only 1.9% of the tetramethyl end group was found. Hirst and Young, however, found no difference when rice starch was methylated in an atmosphere of nitrogen. The results of Hirst and of Hess do not, of course, necessarily apply to methylations in liquid ammonia, but are given to show that a reduction in viscosity is not significant unless it is also shown that the analytical results change concomitantly. A more recent criticism of methylation in liquid ammonia follows from the experiments of Shorigina (74). The excess sodium used in these procedures was found to cause cleavage of methyl cellulose with the formation of desoxy derivatives, which were studied in some detail. Hodge (75) has found that both potassium and sodium amides degenerate methylated starch products in liquid ammonia. This worker suggests that additions of sodium be limited so that an excess is not available for the formation of the amide.

As indicated above, hydrolysis of methylated starch with acid may result in some cleavage of methoxy groups. Accordingly, splitting of the methylated starch by hydrogen chloride in methanol—methanolysis—has been used extensively, the resulting products being the methyl, methylglucosides. In this method, one may suspend the dry, methylated starch in absolute methanol that contains 2.6% hydrogen chloride, allow the resulting solution to stand overnight and then reflux for 8 to 10 hours. Inasmuch as methanol is a product of the hydrolysis of methoxy groups and since this reaction is reversible, the presence of methanol, with the dry HCl as catalyst, should effectively repress the splitting of methoxy groups.

Separation of the methylated sugars is ordinarily accomplished by fractional distillation at very greatly reduced pressure. This procedure affords a ready separation of tetramethyl methylgucoside which distills over first. It is, however, much more difficult to separate the tri- from the dimethyl derivatives. Although determination of the amount of the tetramethyl fraction permits a calculation of the number of terminal groups per unit weight, the result by itself gives little information on starch structure unless one knows the molecular weight of these molecules. The conclusion that branching exists through glycoside linkages follows from a determination of the dimethylglucose fraction. For example, if one obtains 1% tetramethylglucose, the material may be branched, or it may not. If molecular weight determinations show a DP of 100 glucose units, the molecules are not branched; if the DP is 200, then there is one branch per 100 glucose units, if it is 300, there are two branches (three non-aldehydic terminals) and so forth. However, if one finds 1% of dimethylglucose from the methylation of a starch product, then it follows directly that the structure is branched to the extent of one side branch for every 100 glucose units in the structure, and the particular dimethylglucose obtained also indicates the position at which the side branch is attached. A determination of the molecular weight of high polymers is beset with many difficulties and it is doubtful that some of the values previously reported for some of the larger branched molecules are little better than guesses. Improvements in methods to determine dimethylglucose, are, therefore, of extreme importance in the study of starch structure.

Even a determination of the tetramethyl fraction by distillation methods requires the application of a correction factor to the results which may amount to as much as 10% (76). Separation of the several methylated sugars is not complete by usual fractional distillation procedures and the percentages of di- tri-, and tetramethyl methylglucosides in each fraction are usually estimated from refractive index and specific rotation values of the distillate compared to standards composed of known mixtures.

Separation of the several methylated sugars, or their methylglucosides, or other derivatives, may be made by taking advantage of their increasing order of solubility in organic solvents, and decreasing order in water, with increasing number of substituted methoxy groups. MacDonald (77) studied the partition of the di-, tri-, and tetramethyl methylglucosides, and the corresponding aldoses, between water and chloroform. Equal weight mixtures of di- and trimethyl methylglucosides in water were extracted with chloroform. From the residue in water, the dimethyl derivative was recovered in 97.4% yield and from the chloroform extract, the trimethyl, in 97.6% yield. Bell (59, 78) has used a similar procedure. The tetramethyl derivative is even more soluble in organic solvents than the trimethyl. Accordingly, Brown and Jones (79) have been able to separate small quantities of tetramethyl methylglucoside from larger quantities of the trimethyl methylglucoside by extracting aqueous solutions with petroleum ether. It would seem possible, by proper choice of solvents, conditions and apparatus, to develop a quantitative procedure for separating the three classes of methylated sugars found in the hydrolyzate of methylated starch, and it may be that these will give a more reliable value for the dimethyl fraction than can be obtained by fractional distillation. It appears significant that in some instances where separation by solvent extraction has been used (80, 81) less of the dimethyl derivative was obtained than would have been anticipated on the basis of any of our present concepts for starch structure. One cannot neglect the possibility that the high pot temperatures required to separate the tri- and dimethyl fractions by distillation may lead to some scission in the trimethyl derivative with a corresponding increase in the dimethyl fraction.

Neumann and Hess (82) have used a solvent separation technique wherein the methylglucosides are first treated with phosphorus oxychloride. Phosphate groups are introduced into the di- and trimethyl methylglucosides and these compounds are converted into their water insoluble barium salts. The unchanged tetramethyl derivative may then be extracted with chloroform. Coleman and coworkers (83) have treated a mixture trimethyl and tetramethyl methylglucosides with *p*-phenylazobenzoyl chloride (azoyl chloride). The resulting methyl monoazoyltrimethylglucoside may be separated from the tetramethyl methylglucoside in water due to the greatly reduced solubility of the azoylated trimethyl derivative.

Differences in the reactivity of methylated glucose groups, with and without free hydroxyl groups are used to advantage in a new analytical procedure reported by Peat and Whetstone. These workers found (84) that when methylated starch is treated with acetyl bromide, the reaction proceeds with great rapidity at room temperature with a selective removal of the end groups.

Recent efforts to improve the separation of methylated glucoses have been largely through the application, or extension of chromatographic adsorption techniques, Coleman *et al.* (83) have applied aqueous hydrolysis to methylated disaccharides, azoylated the mixture and separated the resulting tetramethylglycosyl azoates from the azoyltrimethylglycosyl azoates by chromatographic adsorption on silicic acid from solution in chloroform. The chromatograms were developed by the addition of benzene which contained 0.1% of ethanol. After mechanical separation of the bands, the azoates were eluted with chloroform which contained 10 to 20% of ethanol. Similar methods have been reported by Myrbäck and Tamm (85) and by Mertzweiller *et al.* (86).

Inasmuch as the methylated sugars are active in ultraviolet light (87), or the bands of these compounds may be seen on adsorption columns after extrusion and streaking the column with a brush dipped into a dilute solution of a reagent such as potassium permanganate, several investigators have applied differential adsorption directly to solutions of the methylated sugars. Jones (88) has separated mixtures of trimethyl methylglucoside (100 parts) from the tetramethyl methylglucoside (5 parts) by passing a solution through a column of Al_2O_3. An equal mixture of ethyl ether and petroleum ether was used as solvent, developer, and eluting medium. Applying this method to methylated rice and banana starches, repeating units of 33 and 26 glucose groups, respectively, were found from end group values. Bell (89) has used silica for the separation of the sugars. Georges *et al.* have studied the separation of methylated sugars on calcium acid silicate (Silene EF) extruding the column and locating the various bands with a light brush mark of alkaline potassium permanganate (90). In the analysis of hydrolyzates of methylated starch, Brown and Jones (79) have proposed a preliminary solvent extraction to enrich the tetramethyl fraction followed by adsorption of the methylglucosides on activated alumina.

The problem here, as in other methods of separation, is the quantitative determination of the dimethyl sugars. From the work in progress in many laboratories on this subject, satisfactory methods, no doubt, will be developed.

The study of the action of enzymes on starch has contributed materially to our knowledge of starch structure and composition. The action is mainly to break starch molecules down into smaller molecular units, the structure of which is known, or may be more easily studied. The scission is hydrolytic in nature and enzymes which promote the hydrolysis of starch are called amylases. The more important of these to this discussion are, alpha-amylases beta-amylases and alpha-glucosidases. The phosphorylases are equally important. They split starch molecules by phosphorolysis, but inasmuch as they also catalyze the reverse reaction, they will be discussed under the separate heading of synthesis.

The amylases are more specific in their action than acid and this, of course, is a distinct advantage in the study of structure. Beta-amylase in particular is very specific in that its active part is apparently able to form a reaction complex only with a maltoside group and then only when this maltoside is connected by 1-4, α-glucosidic linkage to a glucose group. Beta-amylase attacks the non-aldehydic end, or terminals of starch molecules and with unbranched structures, completely hydrolyzes the amylose to maltose by progressively splitting off these sugar units, one after another, down the length of the molecule. With amylopectin molecules, the enzyme splits off maltose units until a point of branching is reached, that is, until the next two units in the remaining structure are not a maltoside group, connected to other units by a 1-4, α-glucosidic linkage.

Those fractions most readily extracted by warm water from some of the common starches such as corn and potato are hydrolyzed to maltose very nearly completely and it is concluded that these molecules are unbranched. Some amylose fractions are hydrolyzed to a lesser extent. Among others, the crystalline amylose prepared from tapioca starch is hydrolyzed to less than 90% of the theoretical yield for maltose (43). This may indicate a minor amount of branching in the structure of this amylose. On the other hand, this low value may be due to a greater difficulty in purifying this very long amylose chain from amylopectin impurities. Some amylopectin subfractions are hydrolyzed to only about 50% of the theoretical yield for maltose. Others are converted to a definitely higher level. Kerr (43) has shown a range in conversion limits between 50 and 60% for corn amylopectin subfractions. These differences in values may indicate differences in the average length of terminal branches, or in the frequency of branching.*

Structural formulas proposed for amylopectin molecules should be in accord with the results of these studies on enzyme action. First of all, some 50 to 60% of the molecule must lie outside a line, or plane, connecting the points of branching in the structure. For example, the structure proposed by Staudinger (56), wherein the terminal branches are, on the average, about 22 glucose units in length and these branches occur every third glucose unit in the central chain, is obviously not in accord with the results of hydrolysis by beta-amylase since such a structure would yield about 85 to 90% maltose. The yield actually obtained requires that these branches should occur at

---

*From a study of corn and sweet potato starches, fractionated with butanol, Hodge, Montgomery and Hilbert (*Cereal Chem*., **25**, 19 (1948)) have recently presented data which indicate the presence of intermediate fractions about every sixteenth glucose unit in the central chain if this type of structure does apply to amylopectin.

Before passing on to more complex enzyme reactions and efforts to elaborate a structure for amylopectin by their use, it should be noted that certain precautions are to be observed in these studies and these apply not only to the use of beta-amylase but to other enzymes to be discussed as well. In the first place it is frequently assumed that a change in reaction rate, or stoppage altogether, is the result of anomalies in structure of the substrate. This is not always the case. Enzymes are relatively labile organic substances, both chemically and physically. Their rate of action is materially influenced by changes in temperature or hydrogen ion concentration and they may be permanently inactivated by extremes of either of these two conditions. They are also inactivated by certain substances commonly called enzyme poisons or they may be inactivated simply by standing under ordinarily favorable conditions if accessory substances are not also present in sufficient concentration. The latter is particularly true of some of the alpha-amylases, but an analogous effect has been noted by the writer when beta-amylase preparations from ungerminated barley grain were highly diluted. A very marked and permanent loss in activity was noted after 5 hours at room temperature.

An equally important consideration is the physical lability of the substrate. Starch molecules associate readily in solution and this is particularly true of the linear fraction. The reaction rate is enormously reduced when amylose retrogrades to an insoluble form and a marked reduction in rate is noted even before insolubilization becomes visible. Since association between either linear or branched starch molecules is difficult to control, considerable care should be exercized in estimating the linearity of these polymers by noting the conversion level at which the reaction appears to stop. In general, high pH and high temperature are unfavorable for polymer-polymer associations as well as, of course, low concentration. All of these should be used at the highest limits which are consistent with the activity characteristics of the enzyme.

Traces of complexing agents which may accompany an amylose preparation, such as fatty acid or higher alcohol, also may affect the rate, or the extent to which the hydrolysis may be carried.

From appropriate tests, it is usually assumed that the enzyme preparation is free from other enzymes which may modify, or extend the reaction under study. With the beta-amylases, the action of which is relatively simple, this condition is not difficult to demonstrate. With more complex systems, such as some of the bacterial or animal alpha-amylases, purity is not so easily shown. There is, apparently some lack of agreement on the functions of these enzymes. Therefore, tests for purity which are based on reactions which the enzyme will, or will not promote, may not be entirely satisfactory. Furthermore, some of these preparations may catalyze reactions, the nature of which is unknown at present. For example, Pigman (91) found that all of the enzyme preparations which he studied, with the exception of malt alpha-amylase and the beta-amylase, are able to synthesize unfermentable substances from maltose. Therefore the possibility exists that if these enzymes act on starch, which largely consists of maltose groups, the unconverted residues during, or after the hydrolysis, are not true sections, or parts of the original starch molecule, but are rather synthetic, in part at least, due to rearrangements which have occurred during the hydrolysis. Indeed, one group of workers (92) recently have actually postulated certain rearrangements in order to explain the action of salivary alpha-amylase according to their proposed structure for amylopectin molecules. The isolation of the amylases in pure crystalline form, the beta-amylase by Balls (93), the pancreatic and bacterial alpha-amylases by Meyer and coworkers (94, 95), and a study of these crystalline amylases on authentic starch fractions should eliminate some of the uncertainties mentioned above and further clarify our conception of their mode of action. This is particularly true of the alpha-amylases.

The action of the alpha-amylases as a class is probably less specific than that of beta-amylase. Alpha-amylase is apparently able to cut off larger sections of a starch molecule than a maltose group and these sections from amylopectin may include a point of branching. This is the initial phase of the reaction during which there is a very rapid decrease in viscosity with the production of relatively few reducing groups. Thereafter these sections are reduced more slowly to smaller units and finally to sugars, depending on the structure of the substrate. There may be some difference in respect to the size of the section first split from the starch molecule by the amylase depending on the source of the enzyme. However, it is apparent that at this stage, points of branching are no barrier to the enzyme. There seems to be some lack of agreement as to whether an alpha-amylase can hydrolyze the anomalous linkages, as well as by-pass them.

Myrback and coworkers (96-170) have extensively studied the action of alpha-amylase and as a result of these studies, favor a multi-branched, or ramified structure (101), similar in its essentials to that deduced by Meyer for amylopectin, to be discussed presently. These results have been adequately reviewed (109-110). Myrback found, in common with other workers, that the reaction consists of two distinguishable phases, the first, a rapid splitting of about 16% of the glycoside linkages with the production of material with reduced molecular weight and called alpha-dextrin. Splitting of the alpha-dextrin proceeds more slowly and practically ends when the DP of the unconverted residues is about 5 to 6 glucose units. Earlier workers, for example, Hanes, found a similar limit (111). In the latter stages of the reaction, some glucose, maltose, and maltotriose are formed. According to Myrbäck, alpha-amylase cannot hydrolyze the anomalous linkage in starch and it will be found in the sugars in the final hydrolyzate. From these, Myrbäck (97) prepared a trisaccharide in which he recognized the presence of a 1-6 glycoside linkage. This result strongly favors the view that starch contains branched molecules and that branching is through 1-6 glucosidic linkages.

Recent studies would indicate that several alpha-amylases do not induce a synthetic action, that their activity is not stopped necessarily by the diminishing size of the substrate, but is limited very likely by anomalous linkages. Bernfeld and Studer-Pecha (112) hydrolyzed an unbranched corn amylose fraction with the crystalline alpha-amylases prepared from pancreas and bacteria and with a highly purified malt alpha-amylase. In all cases the amylose molecule was completely hydrolyzed to maltose and glucose in a ratio of 83% of the first to 13% of the second. Samec and Battestin (113) have reported that potato amylopectin is hydrolyzed to sugar to a significantly lesser extent than the amylose with both malt and pancreas alpha-amylases. Samec and Waldschmidt-Leitz (114) reported complete conversion of potato amylose with pancreatic alpha-amylase.

Hydrolysis of starch in order to obtain sugar units which contain the anomalous linkage in starch has been further studied by Montgomery *et al.* (115). Waxy maize starch was hydrolyzed with an enzyme preparation from aspergillus oryzae, then fermented with yeast and the unfermented sugars purified by treatment with adsorbents. A product was obtained which formed a crystalline disaccharide octaacetate, which apparently is identical to the acetate of the 6, α-D glucopyranoside-β-D glucose prepared by Georges (116) from an entirely different source, dextran.

The action of the alpha-glucosidases on starch has received far less study than the action of the enzymes discussed above. Indeed, with the exception of maltase, such as yeast maltase which splits maltose to glucose, there is some doubt as to whether these enzymes exist as distinct entities. Their existence was postulated by Kerr (117, 118) and others to explain the production of large amounts of glucose when the resistant residues from either a malt or acid hydrolysis were further treated with enzymes prepared from certain microorganisms. Their activity was additionally recognized by the fact that they work best at very high substrate concentrations and relatively high pH and temperature. They are distinguishable from ordinary maltase in the latter two respects. More recently, Corman and Langlykke (119) have reported that some fungal amylase preparations completely hydrolyze starch to glucose. Alpha-glucosidases can, therefore, hydrolyze the anomalous linkage in starch.

Both Meyer and Haworth and their coworkers have used enzyme preparations, which they believed split the anomalous linkage, in order to elaborate further details in the structure for the branched molecules in starch.

Meyer and Bernfeld (120) extracted corn starch with hot water until about 67% by weight remained. This amylopectin fraction was made water dispersible and hydrolyzed with beta-amylase until no more maltose was produced. The resulting limit dextrin (I) amounted to about 45% of the amylopectin. The dextrin was treated with yeast maltase in order to rupture the anomalous linkages which had stopped the action of the beta-amylase. The maltase was allowed to act until 17% of the dextrin had been hydrolyzed to glucose. This value is about twice the calculated amount of glucose which would be produced if the maltase hydrolyzed only one glucose unit from each side chain and if the number of side chains are calculated from the results of end group analysis by exhaustive methylation. The high molecular weight dextrin (II) which remained was again treated with beta-amylase and 20.7% of maltose hydrate was formed, based on the weight of Dextrin I, or 9.5%, based on the weight of amylopectin.

Meyer and Bernfeld explain the renewed activity of beta-amylase after treating the limit dextrin (I) with maltase by a multiple branched or ramified structure for amylopectin, shown schematically in Fig. 66. After hydrolyzing the terminal branches, the activity of the beta-amylase is halted by the first line of branched points. These points of branching are split to glucose by the maltase whereby additional sections of linear structure are opened up for hydrolysis to maltose when beta-amylase is added a second time. The activity of this enzyme is then brought to a halt a second time, when the next line of branched points is encountered and this residue would be dextrin (III).

These data are also thought to show that the laminated structure proposed by Haworth, Kitchen, and Peat is an unlikely one because, when beta-amylase reduces amylopectin to the first limit dextrin stage and maltase is added to hydrolyze the anomalous linkages, then according to a laminated structure theory only a number of linear residues should remain and these should be completely hydrolyzed to maltose by the second addition of beta-amylase.

However, in this line of reasoning the assumption is made that when

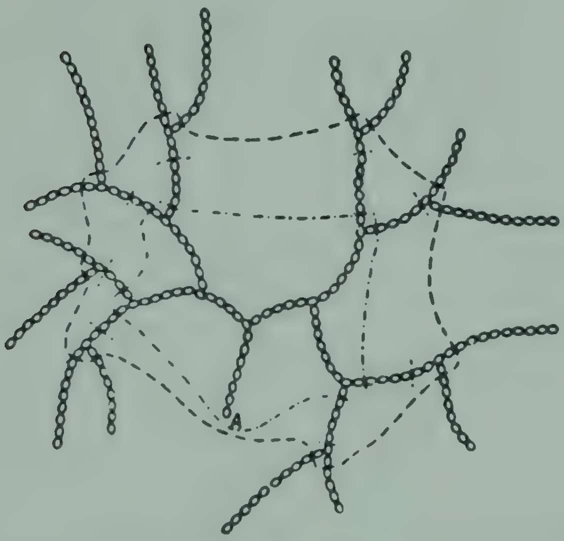


FIG. 66. Ramified structure for amylopectin (Meyer and Bernfeld, 1940).
A, aldehydic end group; o, glucose units.
- - - - End of initial degradation by β-amylase, residual dextrin I.
. . . . . Hydrolysis continued by α-glucosidase, dextrin II.
- . - . End of second attack by β-amylase, residual dextrin III.

maltase is allowed to act on the first limit dextrin, the enzyme attacks all points of branching, more or less simultaneously. Reflection would show however, that this assumption is rather improbable and therefore should be supported by experimental data to demonstrate it. If the amount of enzyme used is limited so that the reaction proceeds at a rate which can be measured and controlled, then it would appear logical to suppose that an enzyme molecule which had made contact with the substrate would not only hydrolyze the anomalous linkage at the point of branching but, being in contact with the substrate would continue on down the length of the substrate structure until there were no more units which it was able to hydrolyze. It seems quite unlikely that after hydrolyzing one 1-6, glycoside linkage, the maltase would then leave this remaining substrate and diffuse to another branch at some distant point. Of course, if a very large amount of enzyme were added so that there was a sufficient number of enzyme molecules to form reaction complexes with each terminal stub simultaneously, then the reaction would proceed in a uniform manner. However, in this case it seems reasonable to suppose that the reaction would proceed too fast to be controlled since very likely, the time involved in these enzymic reactions is due principally to the time required for the enzyme and substrate molecules to make contact and form a reaction complex. The actual hydrolysis is instantaneous, in comparison. All of these suppositions can very easily be shown to be the fact in the case of another starch saccharifying enzyme, beta-amylase. The data of Kerr and Cleveland (40) indicates, for example, that when a limited amount of beta-amylase is added to an amylose solution, then when the enzyme starts to hydrolyze a molecule, it does so completely before another one is attacked. Even after 50% of the weight of the sample has been converted to sugar, the only products recoverable in significant amounts from the reaction mixture are maltose and substantially unattacked amylose. It is possible that a similar mechanism may apply to other enzymes if they can only attack a terminal group on a molecule, and it is possible that the 17% glucose formed from limit dextrin by maltase, in the experiment cited above, may have been derived only from some of the substrate molecules and that the great majority of the dextrin molecules still possessed all of their original anomalous linkages which had blocked the action of beta-amylase in the first place.

The data given in support of the ramified structure shown in Fig. 66 would be more convincing if it were shown that dextrin II yielded approximately the same percentage of tetramethylglucose as was given by the amylopectin in exhaustive methylation procedures, which it should according to the type of structure proposed.

In support of the laminated structure, Haworth *et al.* (92) proposed that when the anomalous linkage in limit dextrin is broken by alpha-glucosidase action, a resynthesis of the same type of laminated structure immediately takes place, again through 1-6, glycoside linkages, but at a different position on the remaining part of the repeating unit chain. Only a part of these unit chains are made available for the further action of beta-amylase. This series of reactions is shown in Fig. 67.

These workers prepared limit dextrin (A) from potato starch by hydrolysis with beta-amylase. The dextrin was treated momentarily with salivary amylase, during which treatment the dextrin was sensitized so that beta-amylase was again able to hydrolyze the substrate into maltose and a second dextrin (B). This dextrin amounted to about 58%, by weight, of dextrin (A), or about 30% of the original amylopectin. Since in the mechanism proposed by Haworth no polymeric bonds (1-6, glycoside linkages) are permanently lost to the structure at the dextrin (B) stage, this 30% residue should yield about three times the amount of tetramethylglucose that is given by the amylopectin, or about 12%. This was found to be the fact.

However, a molecular weight determination on dextrin (B) indicated a DP$_n$ value of 80 and on this basis the value for dextrin (A) should be about 130 and for the original amylopectin, 250. Actually, as pointed out by Haworth the DP of limit dextrin and amylopectin are very much greater, and

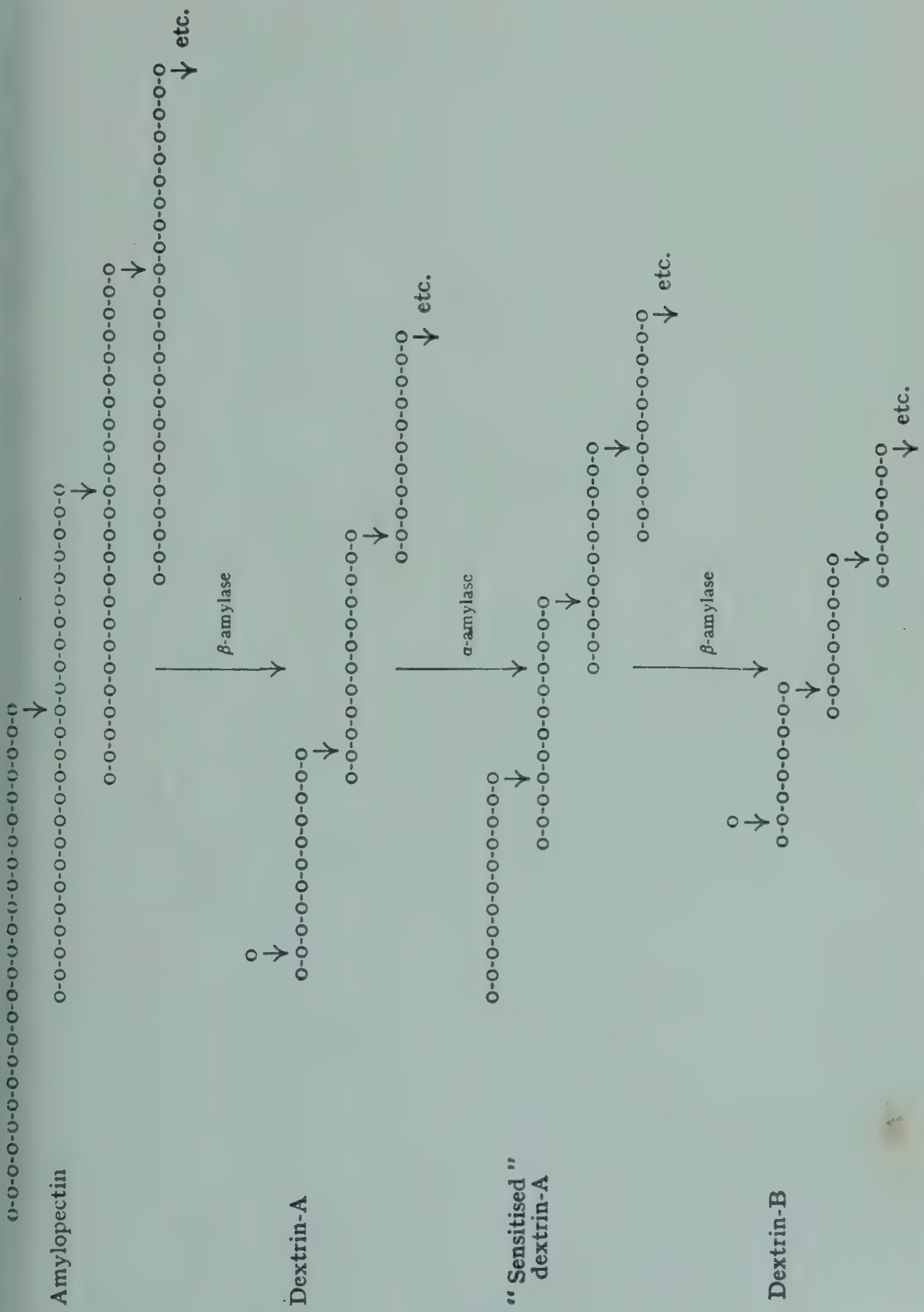

Amylopectin
β-amylase
Dextrin-A
α-amylase
"Sensitised" dextrin-A
β-amylase
Dextrin-B
etc.

FIG. 67. Amylolytic degradation of amylopectin (Haworth, Kitchen, and Peat, 1943).

this suggests another interpretation of the data given by these workers. It would seem possible, that, salivary amylase being essentially an alpha-amylase, acted on dextrin (A) according to the mechanism given earlier for this type of enzyme, splitting some of the molecules at least, into shorter lengths and probably by-passing the original 1-6, glycoside linkages in this section. In view of this possibility, the mechanism given by Haworth and coworkers would be more satisfying if it were shown that the DP of dextrin (A) was substantially unchanged after sensitization by the salivary amylase.

At first thought, the production of rather substantial amounts of maltose (38%) from sensitized dextrin (A) by treatment with beta-amylase would argue against the alternative interpretation suggested. However, it seems quite probable that amylopectin, as a fraction, or as individual molecules does not possess a uniformly branched structure. Some evidence exists, as has already been indicated, that some molecules in this fraction, if not entirely unbranched, at least contain very long sections which are, and these should be more easily approached by enzymes as large as alpha-amylase molecules are now known to be (121). Molecules in which irregularities occur at more frequent intervals would, according to this view, react more slowly. Sensitization would be, therefore, a scission of very large molecules of the first type into shorter lengths from which a large amount of maltose could be produced by beta-amylase, and very little action on the smaller, highly branched molecules.

Haworth has emphasized several times that methylation data give the average value for the length of the repeating units and that some of these units may deviate widely from this mean. Large variations of this nature would explain several of the properties of corn starch, a theory which has been offered several times by the writer. In potato starch there is, of course, the additional irregularity of phosphate groups to be considered.

Haworth, Kitchen, and Peat observed that dextrin (B) was not sensitized by salivary amylase but was hydrolyzed by contact with this enzyme to maltose and dextrin (C), a result which is suggestive of the second phase of a typical alpha-amylase hydrolysis. Dextrin (C), amounting now to no more than about 15% of the original starch, was not acted on by salivary amylase, but was slowly hydrolyzed by pancreatic amylase. A residue was recovered which gave 21% tetramethylglucose, in an end group analysis and which contained all of the phosphorus in the original amylopectin.

It is now known (122) that the activity of the alpha-amylases is related to their ability to form a reaction complex with the substrate and that there appears to be some difference in this respect even among the crystalline enzymes. The view proposed that in this series of hydrolytic reactions, the substrate becomes increasing smaller and irregular, not only directly by hydrolytic scission but also by preferential action on the largest and least irregular molecules, would provide at least one interpretation of the anomalous results reported for the several enzymes in this study.

The action of acid on a dispersion of starch granules is less specific than that of the enzymes. The hydrolysis is less discriminate and eventually all the glycoside linkages originally in the starch are split and glucose is the end product. In addition, side reactions occur under some conditions, particularly a polymerization reaction, in which the glucose units are united through 1-6, glycoside linkages. For these reasons acid hydrolysis has been less useful in structural studies than enzymic hydrolysis. However, there are some data which should be taken into account in elaborating a structure for starch.

Kerr and Schink (117) found that a preliminary hydrolysis of starch with acid did not increase the limit to which malt diastase (a naturally occurring mixture of malt, alpha- and beta-amylases) can degrade starch to fermentable sugars. Indeed, the greater the percentage of glycoside bonds split by the acid, the less the number of bonds the diastase was able to hydrolyze until finally, if starch was hydrolyzed with acid to approximately the same extent as diastase alone is able to hydrolyze the starch, then the diastase was unable to continue the hydrolysis further. These results have been confirmed by Myrbäck and Magnusson (123) and extended by a study of alpha- and beta-amylases separately on acid hydrolyzed starch. Of particular interest is the observation that starch which had been hydrolyzed with HCl to the extent of about 25% yielded the same amount of maltose as did native starch, and no additional maltose was liberated by this enzyme if the acid hydrolysis exceeded 60%.

It may be assumed first, contrary to currently accepted theories, that the attack by acid is not strictly a random hydrolysis, and that a much greater activation energy is required to hydrolyze 1-6, α-glucosidic linkages, and all normal 1-4, α-glucosidic linkages in between points of branching, than the normal linkages in the terminal branches. This view also requires that a large proportion of the anomalous linkages in starch is concentrated in a relatively small fraction, by weight. Little direct evidence exists to support this view and some data have been reported which are incompatible with it. Levine *et al.* (81) fractionated and analyzed acid hydrolyzates of corn starch, and found that they consisted of essentially linear chains. When the starch had been hydrolyzed into the range where the reducing sugars, calculated as glucose, amounted to 40 to 45%, then the dextrins present had chain lengths no greater than about DP 25. The number of end groups found was the same whether calculated from tetramethylglucose determinations or from the acid groups formed by oxidation of the opposite terminals. Furthermore the yield of dimethylglucose was insignificant.

Hirst and Young (71) treated methylated rice starch with oxalic acid in aqueous methyl alcohol and found that there was a rapid disaggregation from a DP of about 1000 to 100. The hydrolyzed product was remethylated in order to derivatize the newly formed hydroxy groups. It was found that the yield of tetramethylglucose was the same before and after the oxalic acid treatment, indicating a repeating unit chain of 31 glucose groups in each case. Had the hydrolysis been brought about by rupture of any linkage other than the 1-6, glycosidic polymeric bond, the yield of tetramethylglucose would have increased with each bond broken instead of remaining constant. Bawn *et al.* determined the activation energy for this initial disaggregation and found that it was 20,700 cal. (124). Although this value is of the order of that required to break a covalent, glycoside linkage, it is considerably less than the 31,000 cal. required to break the 1-4, α-glucosidic linkage in maltose. These data, therefore, are thought not only to support the laminated structure in which the repeating unit chains are joined through covalent bonds, but also to show that, under certain conditions at least, these polymeric bonds are more readily ruptured than the normal linkages in starch.

As a second choice to explain some of the unusual results of acid hydrolysis, one may assume, as did Kerr and Schink, that the anomalous linkages are more numerous than are indicated simply by a study of the extent of branching by end group analysis. However, in view of complete methylation data, as discussed above, it is very difficult to propose an alternate structure which would be entirely satisfactory. Nevertheless, an attempt has recently been made in this direction by Pacsu and Hiller (125, 126). These workers propose that the starch molecule is indefinitely large and that the granule is possibly a visible molecule. It is a three-dimensional network of glucose anhydride units, held together by regular 1-4, α-glucopyranosidic bonds, and periodically these chains are cross-linked through glucose, or longer units, which are joined by hemiacetal or acetal linkages. These glucose units are open in structure and the hemiacetal or acetal linkages are from the aldehyde carbon to carbons number 4, 5, or 6 on other glucose units, in harmony with methylation data. The hemiacetal, or acetal bonds are presumed to be easily split by acid and the two oxygen bridges involved, not necessarily of equivalent strength so that if a 1-4, 1-6 bond starts to split, the 1-4 oxygen bridge may rupture first, thus conceivably giving rise to the 1-6, glycoside linkages which have been found in starch.

Weak acids such as formic, citric, acetic or even $0.1\ N\ H_2SO_4$ hydrolyze these acid sensitive bonds at 80°C. and yield only linear molecules which contain principally normal linkages although 1-6 linkages may also be present in the chains. Indeed, the proportion of amylose obtained in fractionation procedures is thought to be a function of oxonium ion concentration used in preparing the starch sol. Alpha-amylase is thought to be a acetalase whereas beta-amylase attacks the long-chain portion of the starch molecule without liquefaction.

The theory is, admittedly, rather speculative and direct evidence for the existence of hemiacetal, or acetal linkages is required. Halsall *et al.* (127) point out that starch yields one molecule of formic acid per end group when treated with periodic acid and that these results are consistent with a laminated structure but not with the one proposed by Pacsu and Hiller. Pacsu has replied, by inference, that this test is not sufficiently delicate.

When starch, in which all, or part of the original orientation of the granule structure is preserved, is treated with acid, the reaction may follow a different course than when a molecular dispersion of starch is hydrolyzed. When starch granules are treated with acid the initial attack is primarily on the most amorphous regions; the highly oriented linear structures offer a remarkable resistance to attack. Whether or not normal or anomalous linkages are inherently more stable is of secondary importance in this instance. Irregularities in structure which prevent the alignment of molecules will be hydrolyzed in time and linear molecules, or sections of molecules in the crystallites will remain. Thus, when starch is treated with cold hydrochloric acid, as in the production of Naegeli dextrins (128), the end result after many weeks is that only short chain residues remain in the granule structure and these, when dissolved in hot water exhibit the properties of linear polymers of about DP 25. (129).

Some starches such as corn and rice are dispersed with great difficulty. Eighty percent, by weight, of corn starch may be recovered by centrifuging swollen granules from suspensions which have been heated at 90°C. for 1 hour (25). Therefore, even at higher temperatures, at least as high as 80°C., the attack by acid may be expected to follow an irregular course for purely physical reasons.

The very great resistance to acid afforded by orientation of linear polymers may also be noted from the following experiment.* Corn starch samples which had been treated with HCl (0.1 to 0.2 N) for 10 hours at temperatures between 50 and 55°C., were neutralized, dispersed at low concentration in water and fractionated by the addition of Pentasol. The fractions were compared with those of native starch which had been dispersed for fractionation in dilute, cold NaOH solution, and neutralized. It was found from osmotic pressure measurements of the acetates in chloroform that while the Penasol, non-precipitable fraction had suffered severe degradation by the acid treatment to an average DP level of 600, the Pentasol precipitable fraction not only remained substantially at its original DP level of 400 to 500, but also, the percentage by weight of the linear polymer fraction had not decreased below that found in native starch. Furthermore, it was found by dispersing the starch samples in dilute NaOH solution, neutralizing, and titrating with iodine that the percentage of iodine bound by these whole starch samples remained very nearly constant during acid treatment.

---

*Unpublished data.

Therefore, the anomalous effects of acid on poorly dispersed starch, such as the apparent increase in the linear polymer characteristics, and in extreme cases an actual increase in the linear polymer content of the starch, at a very low level of DP, is understandable on a simple physical basis of orientation within the granule through physical bonds. These bonds in starch, such as the hydrogen bond, are resistant to H-ion, but they are readily broken by dilute aqueous alkali, ammonium thiocyanate, chloral hydrate and other dispersing agents.

Several oxidants react in a rather specific manner with carbohydrates and these have been of service in confirming the structures proposed for starch molecules, which have been deduced by other means. These oxidants act on the hydroxyl groups on the second and third carbons in the glucopyranoside units in starch, oxidize these groups to carbonyls and rupture the primary valence between the two carbon atoms. The member of this class of oxidants which has received the most study is periodic acid, and the reaction will be treated in some detail in Chapter XI, on oxidation. Other types of oxidants such as hypochlorite, permanganate, and nitrogen dioxide, very likely react on glucose units in a manner peculiar to each type. However, these oxidations are of a more complex nature, owing to less specificity of the reagent and the difficulty of controlling the reaction. For example, nitrogen dioxide reacts preferentially with primary alcohol groups, where they exist on glucopyranoside structures, as pointed out by Unruh and Kenyon (130), and converts carbon number 6 to a carboxyl group. However, this reagent also oxidizes any free aldehydic, number one carbons to carboxyl groups as well. Since moisture is required to promote the reaction and since water is also a product of the reaction and inasmuch as the oxidant becomes very acidic under these conditions, hydrolytic scission of the labile 1-4, α-glucosidic linkages in starch is extremely difficult to prevent. Each new aldehydic group formed is very quickly converted into a carboxyl. It seems possible, also that during these oxidations there may be some oxidation at carbons 2 and 3.

in the manner indicated for periodic acid. Therefore, in this case, during the reaction we not only have some of the complex structures formed, as with periodic acid oxidation, but also both glucuronic and saccharic acid groups are created and analytical results on structures containing such a variety of groups are very difficult to interpret in a quantitative fashion in terms of structure for the original starch molecules.

Early studies by Malaprade (131), Karrer and Pfaehler (132), Herrissey *et al.* (133), and Jackson and Hudson (134, 135) established the mechanism of the reaction of periodic acid on sugar structures. From these studies and from further work on starch by Jackson and Hudson (136, 137) and Caldwell and Hixon (138) it would appear that if one mole of periodic acid is allowed to react with each glucose group in a linear chain of maltose units, then each glucopyranoside, except the two terminal ones, should be split between carbons 2 and 3 to form a dialdehyde structure. The first two carbons should form a glyoxal group and the other four carbons, an erythrose group. The chain length would be substantially unaltered and would now consist of an alternating series of erythrose and glyoxal groups. The connecting linkages would be acetal bonds involving only one carbon of each glyoxal group. Thus, each erythrose group would be joined on one side through its carbon 3 to a glyoxal, and on the other side through its carbon 2 to another glyoxal.

The terminal glucopyranoside units in starch are further degraded after the primary oxidation. The aldehydic terminal unit yields two molecules of formic acid (from carbons 1 and 2) and one molecule of formaldehyde (from carbon 6), while the opposite terminal unit gives rise to one molecule of formic acid (from carbon 3).

Hydrolysis of the dialdehyde structure and a quantitative separation of erythrose and glyoxal has proven to be a very difficult task. Caldwell and Hixon did separate and identify some quantities of glyoxal both as the osazone and as the benzylphenylosazone and Jackson and Hudson treated the dialdehyde further with bromine water and obtained oxalic acid and *d*-erythronic acid as the brucine salt and as the lactone thus giving qualitative confirmation of the proposed structure. Apparently, the system consisting of aldoses, such as erythrose, and glyoxal not only resists hydrolysis, but also, according to Purves and coworkers (139-142), at least under certain conditions such as methanolysis, the erythrose and glyoxal units may recondense to form a series of cyclic acetals, or hemiacetals.

If a branched structure of glucopyranoside units, such as amylopectin is oxidized with periodic acid, and if branching is through 1-6, glycoside linkages, then the resulting dialdehyde would still consist of an alternate arrangement of erythrose and glyoxal groups. However, in contrast to the dialdehyde from amylose, the structure would be just as branched as the original amylopectin molecule. Furthermore, because there are more terminal groups in a branched structure, more formic acid would be produced. For each branch there would be produced one more molecule of formic acid. Brown *et al.* (143) have attempted to determine the amount of formic acid produced during periodic acid oxidation in order to calculate the extent of branching in starch, glycogen and other carbohydrates. Estimation of the average chain length of the repeating unit in these substances agreed closely with values obtained from the determination of tetramethylglucose in exhaustive methylation procedures. Halsall *et al.* (144) oxidized several varieties of glycogen with potassium periodate and titrated the amount of formic acid produced. Repeating units of 12 glucose residues were calculated, except in the case of rabbit liver glycogen, which gave a value of 18, and again, these results were found to be in harmony with methylation data.

If branching in starch is through linkages to either carbons 2 or 3, and not to 6 as is now assumed to be the case, then when such structures are oxidized with periodic acid, the glucopyranose group at the point of branching would not be attacked by periodic acid. Oxidation of these structures, followed by hydrolysis, should yield one molecule of glucose for every point of branching. Halsall *et al.* (145) have applied this procedure to starches including waxy maize and to limit dextrin, and have analyzed for glucose by chromatographic adsorption technique. Glucose could not be found in any significant quantity thus lending support to the view that branching is not through 1-2 or 1-3 glycosidic linkages, leaving carbon 6 as the only likely point of attachment for branches.

Thus it may be said that periodate oxidation supports the generally accepted theories of starch structure. Admittedly, these data do not possess the weight that is given by other lines of study and it may be said that some, such as the analysis of periodate oxidized starch for glucose, is of a negative character. In respect to the latter it may very well be imagined that failure to isolate glucose, even if it were present, could arise from other causes. For, if branching were to either carbons 2 or 3, and if this structure were oxidized with periodic acid, then a glycoside linkage will remain in this structure connecting the unattacked glucose unit to an adjoining erythrose group. This glycoside linkage will rupture on treating the product with acid, thus splitting the central chain. However, upon examination of the residual structure it is apparent that the unoxidized glucose group is now joined in an aldose glyoxal repeating unit chain in exactly the same manner as the erythrose units are held, which is by acetal linkages to two glyoxal groups, one on one side and one on the other side of the sugar group. Considering the failure of many workers to isolate erythrose in significant amounts from similar structures which should have yielded some 70% of this sugar, the possibility of obtaining glucose, when present in an amount not exceeding 4%, seems rather remote. Following Jackson and Hudson it would seem desirable to treat the product further with hypobromous acid, thus eliminating the glyoxal groups, and analyze for hexose acids such as gluconic or saccharic.

Because the terminal, aldehydic glucose in starch is readily oxidized, several attempts have been made to determine chain length, or molecular weight by this method. The assumption is made that there is only one such terminal per molecule. Here again, the action of periodic acid is rather unique, in that the aldehydic terminal yields among other things, one molecule of formaldehyde. Caldwell and Hixon (80) calculated the chain lengths of a series of fractions from beta-amylase limit dextrin by the amount of formaldehyde liberated in periodate oxidation and found that the values were in agreement with the reducing values of the dextrin fractions.

Many oxidants of low potential such as hypoiodite (80, 81, 146) copper salts (147) and silver nitrate (23), in basic media, oxidize the terminal aldehyde group in starch, and have so been employed as an end group procedure to measure the chain length, or molecular weight of the starch preparation. However, the difficulty which arises from the use of procedures of this nature is that the reaction may not be stoichiometric and may proceed further with the carbohydrate than has been assumed. However, in the case of hypoiodite oxidation, Hixon and coworkers found a close agreement in the amount of iodine consumed and the number of carboxyl groups produced presumably terminal ones over a DP range of 25 to 125. Dextrins produced from enzymic and acidic hydrolyses were examined. Richardson and coworkers oxidized starch in alkaline dispersion with copper salts and found an average DP in the neighborhood of 1000. Meyer and coworkers oxidized a corn amylose sample (hot water extracted) which showed one non-aldehydic terminal per 300 glucose units in a methylation end group analysis. Silver nitrate was added to an alkaline solution of the amylose and the results showed one oxidizable terminal for every 300 glucose units. Since osmotic pressure measurements also gave the same value for DP, the results on oxidation may be taken as further confirmation that the structure is linear.

The value of the reaction, in which a blue colored complex is formed between amylose and iodine, to the study of starch structure and composition need hardly be discussed further at this point. There is a remarkable difference in the behavior of amylose and amylopectin fractions in respect to their ability to form an iodine complex and this can satisfactorily be explained on the basis of a difference in structure. Rundle, French, and their coworkers have admirably accounted for this difference by the concept that mobile, linear starch chains, of necessary length, can form a helical coil around a sufficient number of iodine molecules, whereas short sections of a branched, or ramified structure cannot. This reaction will be discussed in some detail in Chapter XVII. In view of the convincing evidence given by these workers, which has established the configuration of the starch-iodine complex, the requirements in respect to configuration of the starch molecule in order to enter into complex formation (29, 148-151), and the nature of the reaction involved (28, 46, 152), it may be said that this study affords very strong confirmation of our modern basic theory as to the composition and structure of starch.

The iodine reaction shows promise of being of considerable value in fundamental studies as a tool to characterize, chemically, starch fractions. Among the unbranched polymers, one may obtain from potentiometric titration or spectrophotometric determinations the relative, average DP, or chain length, of the samples, and from the former, the molecular weight distribution (153). Among the branched polymers one may determine the relative frequency of branching. However, although methods have been very highly developed to follow the formation of complex between iodine and starch, correlation of data in terms of structure in a more quantitative manner has been retarded for one or more of the following reasons: Lack of (a) standard samples with a satisfactory narrow range of chain lengths, (b) precise procedures to establish whether or not the samples are completely unbranched and (c) acceptable molecular weight data on the standards used for calibration.

The last named deficiency has handicapped many of the studies on structure discussed in this section and has made more difficult a quantitative interpretation of results. Because of the very great size of starch molecules, their effects are extremely feeble when one attempts to measure them in molecular weight determinations which work so well with substances of low molecular weight. Classical procedures such as, elevation of the boiling point and depression of the freezing point of suitable solvents are entirely too crude for this purpose and one must resort to more complicated methods such as sedimentation and diffusion rates, light scattering data, osmotic pressure and viscosity determinations, wherein the effect of solute particles can be measured with greater precision. Even so, and with the best equipment, reproducibility of results within 5% is exceptional. The actual, or inherent error in many of these procedures is quite unknown and may be many times larger. One contributing factor is because in all physical methods for the determination of molecular weight of starch, measurements are made on solutions of the polymer and calculations are based on the assumption that the solute is molecularly dispersed. It is so very difficult, if not impossible, to show whether they are, or are not, that the basis for the assumption is usually neglected entirely. If an optically void dispersion is obtained the presence of only discrete molecules is usually taken for granted.

The association of starch molecules in neutral aqueous media is notorious. For this reason starch is derivatized to form an ester or an ether and organic solvents are employed to prepare solutions for testing. In this manner the very great tendency of starch molecules to associate through their hydroxyl groups (as for example, by hydrogen bonding) is blocked. However, it seems fairly evident that these derivatized molecules may associate to such an extent that some solvents, even with thermal activation, cannot completely dissociate these complexes. Staudinger (56) made a very serious effort to show that the very large molecular weights obtained for starch, as from osmotic pressure measurements were real and that starch was not simply an aggregation of very small molecules, a theory which had prevailed during the decade prior to his work. Starch was acetylated. Part was deacetylated and another part was converted into the methyl ether. Osmotic pressure measurements were made on the starch, the triacetate, the deacetylated ester and the methyl ether and the molecular weights were found to be the same, approximately, in all cases. Staudinger concluded that an associated complex, such as a micelle from the native granule, could not be expected to persist as such throughout these several chemical manipulations, and of course it does not seem likely that it could. However, these, or similar experiments, do not necessarily prove that although original associative bonds are destroyed, either in whole or in part, the newly formed derivative cannot reassociate through newly formed bonds. Since it is now known that the molecules in starch, although probably very large, are nevertheless associated and since it is fairly evident that they do not dissociate, or remain dissociated, completely in such solvents as water, and possibly formamide, one would naturally expect that if the esters or ethers were completely dissociated in solution, then lower molecular weight values would have been obtained for these derivatives.

If one observes the reasonable order of viscosity of corn B-fraction in hot water, adds pyridine, and distills, as in the method of Pacsu to prepare the material for acetylation, one cannot fail to be impressed by the tremendous physical change which is initiated when acetic anhydride is added and the acetate starts to form. If native corn B-fraction is present in concentrations of the order of 0.5 to 1.0%, or higher, in the reaction medium, the acetate "solution" will set to a gel when allowed to stand at room temperature. Even at concentrations of 0.5% B-fraction, the reaction medium containing the triacetate is scarcely fluid at 100°C. If the solvents present are removed by washing with water, the dried product is substantially insoluble in all neutral solvents although it will swell to form a gel in chloroform which is one of the better solvents for amylose acetate.

Foster and Hixon (154) reported that the very large potato amylose acetate molecules tended to form a gel in chloroform. The acetates of corn and tapioca amyloses (by butanol fractionation) gave osmotic pressures in this solvent corresponding to DP_n values of 260 and 460 respectively. Potato amylose was estimated to have more than twice the DP of corn amylose from comparative alkali lability values.

Derivatives of either linear or branched starch molecules, with DP values less than about 600 to 800 appear to form solutions in some solvents, but even here, one may obtain very different DP values depending on the solvent used and the conditions employed to prepare the solutions. The acetates appear to give the lowest osmotic pressures when dissolved in chloroform near the boiling point, and for the present it may be assumed that these are nearest to the true values for molecular dispersions. Osmometrically determined molecular weights for fractions which are composed of very small starch molecules, *e.g.*, DP of 100 to 300, are quite possibly true ones since these are in agreement with results calculated from chemical end group analyses (23), and since there cannot be less than one end group per starch molecule.

A minimal molecular weight value is, of course, not necessarily a criterion of a correct result. Considerable care should be exercised in the preparation of derivatives so that the polymer is not degraded during derivitization. Reactions which require the use of high temperatures or highly alkaline or acidic reaction media are to be avoided with starch. For example, it might be anticipated that methyl ethers, prepared by such common procedures as repeated treatments in 5% NaOH solutions at 50°C., or nitric acid esters, prepared by the usual methods of treatment with nitric-phosphoric acid mixtures, are not particularly suited for molecular weight determinations. The fatty acid esters, prepared by the addition of the acid anhydride in relatively neutral reaction media such as pyridine, are probably less degraded than other derivatives. However, many starch acetates, particularly of B-fractions of high molecular weight, when prepared by methods involving dispersion in aqueous pyridine, are, as intimated above, so highly associated that they will not disperse molecularly in any neutral solvent. Acetates

TABLE XIV

Molecular Weights of Starch Fractions, as DP

	Samec[a] Osmotic Pressure in water	Fox (163) Ultra-centrifuge, acetates in methyl acetate	Dumbrow (161) Ultra-centrifuge, acetates in methyl acetate	Horan (47) Ultra-centrifuge, acetates in methyl acetate	Foster and Hixon (154) Osmotic pressures, acetates in chloroform	Caesar *et al.*[b] Isopiestic method, nitrates in ethyl acetate	Meyer, *et al.*[c] Osmotic pressure acetates in tetra-chloroethane	Kerr, *et al.* (42) Osmotic pressure, acetates in chloroform
Amyloses								
Corn					260	220		455
Hot water extracted		540	375		175		270	225
Tapioca					460			1050
Hot water extracted			525					
Potato	770			1850- 3700		980		850[e]
Amylopectins								
Corn								1450[e]
Hot water res.		20,000- (w) 50.000[d]					1300	
Tapioca				6000(*w*)[d]				1300[e]
Potato	1450							

[a] *Z. physiol. Chem.*, **259**, 204 (1939); **267**, 243 (1941).
[b] *J. Am. Chem. Soc.*, **69**, 617 (1947).
[c] *Helv. Chim. Acta*, **23**, 885 (1940).
[d] (*w*) = in water.
[e] Unpublished results.

which are obviously more soluble in solvents such as chloroform are prepared by dispersion of the starch fraction in formamide, followed by acetylation with the anhydride and using pyridine as catalyst. The osmometrically determined DP_n of corn amylopectin acetate, prepared by the latter method was found to be 1450 and that of tapioca, 1300 (31), as shown in Table XIV.

The considerations discussed above apply not only to osmotic pressure measurements but also to other physical methods for determining the molecular weight of starch since they too involve measurements on solutions. These considerations very likely account, in part at least, for the very great variation in molecular weights of native starch fractions reported by different workers, some of which are shown in Table XIV.

Viscosity measurements are performed so easily and quickly that these procedures do not suffer some of the limitations of other physical methods. One may, for example, use starch fractions, without derivitization, in strong dispersing agents such as anhydrous ethylenediamine or KOH solution at room temperature whereas, these solvents may be difficult to use in equipment employed in other methods. However, it has become apparent that intrinsic viscosity is not one simple function of molecular weight, but that it varies with the polymer-solvent system and that the viscosity for an average DP is inordinately affected by the molecular weight distribution and by the shape of the polymer. Therefore, for any particular system, such as, unbranched amylose molecules in aqueous KOH solution, it becomes necessary to determine the function between intrinsic viscosity and molecular weight by some independent method of measurement of the latter on carefully fractionated samples, before viscosity can be used to determine the DP of an amylose of unknown molecular weight.

On the other hand, one may use viscosity data to show differences in structure, qualitatively at least, between starch fractions. Huggins has shown (155, 156) that the constant, k, in the equation

$$\mathrm{k} = \frac{\eta_{sp}/C - [\eta]}{[\eta]\,\eta_{sp}}$$

serves to characterize a solute-solvent system and that the value is the same for all members of a polymer series irrespective of molecular weight, and, accordingly independent of molecular weight distribution. η_{sp} is the specific viscosity, C is the concentration and $[\eta]$ is the intrinsic viscosity. Kerr (43) determined the viscosity of corn amylose and amylopectin subfractions in anhydrous ethylenediamine and found, in general, a different range of k values for amylose and amylopectin, thus confirming the view that the polymer is not the same in both instances, but differs in structure. Independently, Speiser and Whittenberger (157) found a wide difference in k values for potato amylose (precipitated by nitrobenzene) and potato amylopectin subfractions when dissolved in dilute aqueous KOH. These workers concluded that this test may be used as a general one to detect branching in high polymers. Bartovics and Mark (158) had applied the procedure for this purpose in a study of the polymerization of styrene at different temperatures.

A very large number of experiments (159) confirm the conclusion that the viscosity of a solution increases with the axial ratio of the solute, other conditions being constant. The data of Speiser and Whittenberger show that all potato amylose subfractions are very much more extended in shape than potato amylopectin subfractions, which supports the view that the amylose is linear and thread-like whereas the amylopectin is more compact in shape such as might be expected of a branched structure. However, Kerr found that whereas some corn amylopectin (butanol non-precipitable) subfractions had relatively small intrinsic viscosities in ethylenediamine, and glycogen gave almost an insignificant value of 0.1, one amylopectin subfraction showed an intrinsic viscosity very much higher than any of the amylose subfractions. From this it was concluded, as mentioned earlier, that some of the molecules in corn starch which are not precipitable by butanol are more extended in shape, and in this sense more linear, than those in the butanol precipitable fraction.

The shape of solute particles is also indicated by the value for the exponential constant, a, in the equation relating intrinsic viscosity to molecular weight, M, as follows:

$$[\eta] = b\, M^a$$

The calculations of Huggins (160) and others have shown that a has a limiting value of 2 for long rigid rods and a value of one, or less, for randomly kinked chains. The minimum value is 0.5. Crystalline amyloses from corn, potato and tapioca starches (hot water extracted, butanol precipitated) when converted into their acetates by Dombrow (161) gave a value of a 0.97 in methyl acetate. M was determined from sedimentation-diffusion data. Subfractions of corn amylose (butanol precipitate) likewise show a value less than unity when their viscosities in ethylenediamine are compared against their osmometrically determined molecular weights, using the acetates in chloroform solution (see Table XII). Horan (47) compared a series of HCl degraded potato amylose fractions (butanol precipitates) against a similar series of potato amylopectin fractions. M was determined from sedimentation-diffusion data. For the amylose acetate fractions in chloroform a was found to be 0.72, and in methyl acetate, 0.57. On the other hand the amylopectin fractions gave a value for *a* of 1.3, indicating that their structure is more rigid. This value approaches that determined from theoretical considerations by Simha (162) for a prolate spheroid. Quite possibly a laminated structure with polymeric bonds periodically spaced along the principal axis could fulfill the approximate requirements for shape and supply the necessary rigidity.

Beckmann and students (47, 161, 163) have studied starch and starch fractions in the ultra centrifuge. Distribution diagrams show the presence of two widely separated groups in starch, one of low and the other of high molecular weight. In corn starch, occasionally, the presence of a third, or intermediate group was also indicated. Thus, heterogeneity in starch is confirmed. Molecular weights determined by this method for several fractions are also given in Table XIV.

Using molecular weights determined from sedimentation-diffusion data and axial ratios from viscosity, Dumbrow (161) found that the acetates of the crystalline amyloses of tapioca, corn and potato starches possessed a configuration in methyl acetate with a length some 25 to 40 times greater than their diameter, and are accordingly linear polymers. The average diameter of these dissolved molecules were found to be the same, regardless of source, and approximately double those of methyl cellulose of corresponding DP. This difference was explained as being due to the more flexible nature of the amylose molecule and the tendency of the derivative to form a helical coil in this solvent.

Rundle and Baldwin (148) were able to orient potato amylose molecules in solution under the influence of a velocity gradient, sufficiently to show dichroism of flow, whereas potato amylopectin (butanol non-precipitable fraction), waxy maize and glycogen could not. Support is thereby given to the conclusion that the amylose is linear in structure whereas the potato amylopectin, waxy maize and glycogen molecules are much less asymmetric in shape, such as would be the case if these latter possessed a highly branched structure. A similar conclusion follows from the observation made by many workers that amylose derivatives form self supporting films and may be drawn into fibers, as would be expected of linear polymers whereas amylopectin derivatives form brittle films and cannot be drawn into fibers.

Therefore, it may be concluded that the data reviewed in this section, and considered together in its entirety, convincingly support the general theory for the structure of starch molecules which was outlined in the introduction.

4. Non-Carbohydrate Constituents of Starch. Whether or not starch is pure carbohydrate has been the subject of extensive research

It has been proposed that, in addition to the glucose residues of which the molecules of starch are composed, there are other substances combined with them. Various theories have been developed from this assumption to explain certain anomalies in the chemical and physical behavior of the starches.

It has been shown by analysis that when various starches are purified by non-hydrolytic agents such as water at low temperatures and ether and then are completely hydrolyzed by dilute hydrochloric acid, appreciable amounts of phosphates, fatty acids, nitrogenous bodies, silica, and other substances may be recovered. The results of analyses by Samec and Blinc (13), Edwards and Ripperton (164), Taylor and his coworkers, and others, for some of the common starches are shown in Table XV. Both potato and wheat starch yield considerable amounts of phosphate; corn yields fatty acids, amino acids, and occasionally silica; rice and wheat yield nitrogenous material, and so forth. Inasmuch as all of these substances possess acidic functions (the amino acids, basic functions as well), it might be presumed that some of the many hydroxyl groups present in the fundamental starch molecule are esterified with these acids. If such were the case, then molecules esterified with phosphoric acid might be expected to be more lyophylic, and an esterified glucopyranose unit might be expected to present a barrier to the systematic and highly specific activity of enzymes, such as, beta-amylase. Molecules esterified with fatty acids might be expected to be more soluble in alkaline solution, but tend to become insoluble, or be originally insoluble,

TABLE XV

Some Non-Carbohydrate Constituents of Certain Starches[a]

The values are given in per cent.

	Potato	Wheat	Corn	Rice	Sago	Tapioca	Arrowroot
P_2O_5	0.176	0.149	0.045	0.015		0.017	
P_2O_5[b]	0.287	0.265	0.069	0.243	0.054	0.062	0.023
SiO_2	0.069	0.019					
SO_3	0.008	0.066					
CaO	0.058	0.042	0.024	0.014	0.025	0.015	0.112
MgO	0.001	0.026	0.014	0.040	0.005	0.005	0.003
K_2O	0.018	0.027					
K_2O[b]	0.072	0.087	0.028	0.063	0.082	0.024	0.028
Na_2O	0.008	0.032	0.041	0.283	0.032	0.027	0.020
Fe_2O_3	Trace	Trace					
N	0.0113	0.0488					
Fat[c]	0.04		0.61	0.83	0.11	0.12	

[a] The table is compiled principally from data from the works of Samec and Blinc and of Taylor, referred to in the text, and from the report of Edwards and Ripperton.
[b] Edwards and Ripperton used native starch for the analysis.
[c] Difference between total fat and ether-soluble extract.

in neutral or acid solution. Combined silicic acid might be expected to confer a measure of insolubility on starch fractions or a tendency to form gels in neutral or acid media, which physical states would tend to resist enzymic attack. The literature on this phase of starch chemistry is very extensive, not only in experimental evidence to support the theories given but also on the alternative side of the question. Inasmuch as many of these questions have been definitely settled in recent years to the effect that with only a very limited number of exceptions starch is now known to be pure carbohydrate, the subject has lost much of its former importance. Hence only a brief reference will be made to sources in which the theories and data pertaining to this discussion have been more completely treated.

Samec (12) has discussed the role of phosphate in potato starch. The early work of Northrop and Nelson (165) showed that, by progressive hydrolysis of potato starch to products of very low molecular weight, fractions could be separated from the hydrolyzate which had progressively higher phosphorus contents. Sherman and Baker (166) extracted potato starch with hot water and showed that the extracted solids contained only 0.023% P_2O_5 whereas the swollen residue contained 0.147%. The fractionation experiment was extended by Baldwin (20) and the uneven distribution of phosphorous between soluble amylose and the residual amylopectin confirmed. Kerr and Severson (24) observed a very unequal distribution of phosphorus between the fraction of potato starch precipitable from aqueous solution by the addition of alcohols and the fraction not precipitated, but not in the case of corn starch. Kerr found no phosphorus in the amylose crystallized from hot water extracts of potato starch by the addition of butanol.

As mentioned previously, Samec ascribed the ability of amylopectin in potato starch pastes to move to the positive electrode in an electrophoretic cell to esterified phosphoric acid groups which are ionized to anions and confer a negative charge on this carbohydrate fraction whereas amylose does not migrate because it contains no phosphoric acid. Samec further proposed that many of the differences in colloidal behavior between amylose and amylopectin, particularly their viscosity characteristics, were due to phosphoric acid groups in the amylopectin fraction.

Posternak (167) extended the experiments of Northrop and Nelson. Using a combination of enzymic hydrolysis of potato starch to arrive at a tetrose which contained a phosphate group followed by acid hydrolysis which yielded finally glucose-6-phosphoric ester, it was demonstrated that the phosphate in potato starch is esterified with glucose units at carbons number 6. Robinson and King had isolated this glucose ester from the end-products of starch fermentations.

Wheat starch, as well as corn starch yields as a final product alpha- and beta-glycerophosphate. Furthermore, from the fact that both the phosphorus- and nitrogen-containing bodies may be removed by extracting gelatinized wheat starch with alcoholic solutions, although potato starch so treated is not materially reduced in respect to its phosphorus content, Posternak concluded that the phosphorus in wheat starch is present as a phospholipid and as such is present as an impurity or as a simple physical mixture. Schoch (168) has shown that when wheat starch granules are extracted in a Soxhlet extractor for 48 hours with 80% aqueous dioxane, the phosphorus content is reduced from 0.054% to 0.008%, whereas the phosphorus content of potato starch granules was reduced only from 0.095% to 0.087%. It is evident, therefore, that phosphate does exist in chemical combination with some starch molecules, in particular potato amylopectin and possibly amylopectins from other starches obtained from roots and tubers.

Briggs and Hanig (169\ found that titration curves on exhaustively electrodialyzed potato starches showed buffer ranges which correspond with those of the first and second hydrogens of orthophosphoric acid. That corresponding to the first hydrogen agreed well with that expected from the total phosphorus content of the starch. Titration curves for exhaustively electrodialyzed wheat and corn starches, however, showed no buffer capacity in the range of pH in which the first hydrogen of potato starch is titratable. Some buffer capacity was noted in higher pH ranges but this was attributed to the fatty acids in the cereal starches.

It has been known for many years that some starches contain fatty material in such close association that they cannot be purified from these constituents by mechanical or physical means in starch manufacture. Starch factories which hydrolyze starch with acid to produce sirups and sugars must remove considerable quantities of an insoluble "refinery residue" in the clarification of these fluid hydrolyzates. In the use of corn and some other starches, this residue contains substantial amounts of fatty acids. More than fifty years ago, Sostegni (170) hydrolyzed rice starch and showed that the insoluble residue which remained contained a fatty material, soluble in ether, which melted at about 48°C. Taylor and Nelson (171) and Taylor and his students (172-176) studied the fatty material associated with corn starch. After the starch was thoroughly purified, including repeated extractions with ether, it yielded about 0.6% of fatty material after hydrolysis, and in this residue palmitic, oleic, and linolic acids were identified. Lehrman (177) found the same acids in tapioca starch, and in addition, linolenic acid. Lehrman and Kabat (178) could find no fatty acids associated with potato starch, however.

At first it was believed that these fatty acids were esterified with glucose units in one fraction of starch. Since this fraction, called alpha-amylose by Taylor, migrated in an electrophoretic cell it was believed to be comparable to the amylopectin-phosphoric acid ester of potato starch, which is separated from potato amylose by this procedure. Taylor and coworkers called the residual starch fraction which was free of fatty acid and which did not migrate, beta-amylose.

However, Kerr and coworkers (179) concluded that beta-amylose is not amylose but has a limit of conversion to maltose with beta-amylase approaching more nearly that of amylopectin.

Rask and Phelps (180) showed that the fatty acids in corn starch could be completely extracted with alcoholic ammonia. Schoch (168, 181) found that aqueous methanol and dioxane solutions removed the fatty acids from corn, wheat, and rice starches and Lehrman (182) concluded that the fatty acids present in starch are adsorbed.

Kerr and Trubell (26) found that native corn starch showed a much lower amylose content by iodometric analysis than defatted corn starch and concluded that the amylose, in part at least, was insolubilized by the fatty acids, rendering this fraction less able to orient with iodine. Kerr (25) also showed that by defatting corn starch the solubility of amylose was increased to the extent that the amount which dissolved in water at 70°C. increased nearly threefold. Schoch and Williams (183) found that fatty acids precipitate amylose from dilute starch sols and may be used as fractionating agents. Mikus *et al.* (184) studied the complex formed between amylose and fatty acid and reported that it was quite similar in configuration to that of the amylose-iodine complex. Even a prolonged treatment with carbon tetrachloride did not remove the fatty acid which is held by dipolar interactions within helicies formed by amylose molecules. These workers determined the stoichiometry of the reaction for several fatty acids and amylose. In the resulting complexes it was found that there were 17.6 glucose residues for each lauric acid molecule, 22.6 per palmitic and 25.0 per oleic acid molecule, respectively. These ratios are proportional to the lengths of the fully extended fatty acid molecules, including van der Waals distances, which are, 19 A. for lauric, 20 A. for palmitic, and 27 A. for oleic acid. Furthermore, it was found that when amylose in the helical configuration was suspended in methanol solutions of palmitic acid, of various concentrations, the amylose was able to combine with as high as 4% by weight of fatty acid, whereas retrograded amylose and amylopectin adsorbed only 0.1 to 0.2% by weight of fatty acid over the same range of concentration. The amylose used in these studies was prepared from lily bulb starch by precipitation with butanol and was found to have about 90% "purity" by iodometric analysis, using the corn crystalline amylose of Kerr and Severson as a standard.

Therefore it may be concluded that the amylopectin of some starches is not esterified with fatty acid, as originally believed, but rather that amylose and fatty acid may unite to form a molecular complex which, under certain conditions approaches the constancy of composition of a reaction compound.

Clayson and Schryver (185), Schryver and Thomas (186), Ling and Nanji (187), and others have observed that some starches, particularly the cereal starches, appear to contain a component resistant to the action of ungerminated barley diastase. Ling and Nanji analyzed this fraction and consistently found about 1% SiO_2. They concluded that the product was a salt of a silicic acid ester of amylose and that the resistance to enzyme action could be explained on this basis.

Samec purified starch (188) by treating it with a solution of hydrofluoric acid, which removed the silica. However, the same amount of enzyme resistant fraction, about 10%, was obtained from both the purified and unpurified starches.

Kerr and Trubell (189) prepared this resistant fraction from corn starch but found only 0.15% of SiO_2 in the product. Purification of the fraction was made by dissolving it at 25°C. in 0.67 N NaOH, centrifuging and precipitating the amylose from the centrifugate by adjusting the pH to 5.0 with HCl. After washing with water and drying, this product was found to contain less than 0.04% SiO_2. Silica is evidently an impurity in starch which, when gelatinized in hot water, yields an insoluble residue, particularly in the case of cereal starches; the silica collects with the insoluble, or less soluble fractions. The resistant fraction is very likely closely related to that subfraction of amylose which remains undissolved when starch granules are treated with water at 90°C., and owes its enzyme resistance to its insolubility.

It is extremely difficult to purify some starches, *e.g.*, corn and wheat, from the last traces of nitrogenous bodies. In early work Samec proposed that the nitrogenous material was of a protein nature and formed a salt with the dibasic amylopectin-phosphoric acid molecules. Certain differences in colloidal behavior, conductivity, and titration characteristics, which exist between wheat starch and potato starch preparations that contained no protein were thought to be explainable on this basis. However, Samec (190) concluded from later work that the theory of chemical union of the protein through salt formation was not entirely satisfying. Samec and Blinc (13) proposed that a union of starch and protein exists through coacervation. This phenomenon, studied in the many researches of Bungenberg de Jong

(191), has also been accepted by Koets (192, 193) and by von Przylecki and coworkers (194) to explain the physical, colloidal union between starch and protein. Dobry (195) has recently discussed the nature and function of coacervates between proteins and carbohydrates.

5. Synthesis. Additional information concerning the chemical nature of starch and its relation to glycogen is obtained from studies on the action of the enzyme system, phosphorylase, which catalyzes the reversible reaction: starch (or glycogen) + inorganic phosphate $\rightleftharpoons$ glucose-1-phosphate. Cori and Cori (196) have reviewed early experiments on the study of the enzyme, which apparently is widely distributed in nature. It was originally shown to be present in extracts of muscle, heart, liver, brain, and yeast. Hanes (197, 198) has reported its presence in a variety of plant tissues, particularly potatoes which are apparently an excellent source. The nature of the enzyme and the kinetics of the reaction have been studied by Cori and Cori (199, 200) and by Hanes (201). According to Cori, the reaction, regardless of whether plant or animal phosphorylase is used, is similar in many respects. There is an initial lag period before the enzyme develops activity in the liberation of inorganic phosphate and the synthesis of products with the characteristics of glycogen, starch, or its fractions. This induction period becomes extended indefinitely as the enzyme is purified, but may be eliminated by the addition of glycogen, starch or some of their hydrolysis products, which are apparently necessary and enter into the reaction, in a manner which will be discussed later. The position of the equilibrium of this reversible reaction is influenced by the pH. Cori found that whether equilibrium is approached from one direction or the other, the ratio of inorganic phosphate to glucose-1-phosphate is 5.7 at pH 6, but falls off to 2.7 at pH 7.6. Hanes and Maskell (202) confirmed the effect, using potato phosphorylase, and found a ratio of 10.8 at pH 5.0 and a ratio of 3.1 at pH 7.0. Temperatures between 15 and 30°C. have no effect on the position of equilibrium. The time required for the reaction to reach equilibrium in the presence of low concentration of activator depends on the enzyme concentration. Although the addition of activators, such as glycogen or starch molecules, increases the reaction rate, it does not affect the equilibrium point unless there is an insufficiency of activator, in which case the reaction rate may be very greatly reduced. In some cases equilibrium may not be attained even in a very long period of time.

From these and other considerations, particularly a study of other phosphorylase catalyzed reactions, Cori *et al.* (203) proposed that the synthesis is a bimolecular reaction in which the activating polysaccharide is one of the participants. Thus, glucose-1-phosphate does not react with itself to form a carbohydrate chain but rather with the non-reducing end of a carbohydrate molecule which must be supplied and the reaction consists of a lengthening of these original molecules. However, if there is a sufficiency of activating nuclei, in relation to active enzyme groups, then, kinetically the reaction will be of the first order. Furthermore, since the concentration of terminal glucose units remains constant, even after reaction with the phosphate ester and the addition of new glucose units, it is evident that the equilibrium point is not dependent on one concentration of added activator in this case either.

Glucose or even maltose are not suitable activators, or participants, for this reaction. Potato phosphorylase can, however, be activated by relatively short polysaccharide chains, possibly of the order of 5 to 7 glucose units in length, since partially hydrolyzed Schardinger dextrins activate this enzyme. Crystalline muscle phosphorylase requires the presence of a larger molecule. This difference between phosphorylases, depending on source, recalls differences between the pure alpha-amylases, which were discussed earlier, and would lend support to the theory, originally proposed by Cori, that the first phase of the reaction is the formation of a reaction complex between the activator molecules and the enzyme.

The above given mechanism would explain why polysaccharides with many comparatively short branches in their structure, such as amylopectin and glycogen, have a much greater activating effect per unit weight than long, linear molecules such as amylose, and it would explain why the linear polysaccharide formed by phosphorylase itself has no activating effect during the course of the reaction (203, 204). Also consistent with the viewpoint given is the observation of several workers that whereas a limited acid hydrolysis of branched amylopectin and glycogen molecules increases their activating power to a measurable extent and is then followed by a decrease with further hydrolysis, the hydrolysis of amylose, either with acid or with alpha-amylase very materially improves the activating power per unit weight. In the case of amylose, the number of terminal groups, which may act as nuclei in the synthetic reaction, increases materially per unit weight as these hydrolyses proceed. It is interesting to note however, that when amylose is hydrolyzed with beta-amylase, the activating power declines as the hydrolysis proceeds (203). This result favors the view proposed by Cleveland and Kerr (40) that the action of a beta-amylase group is to hydrolyze completely each amylose molecule before starting the attack on another, rather than a simultaneous attack and shortening of all amylose molecules present.

Cori *et al.* (203), in a study of the action of crystalline muscle phosphorylase, found that a limiting factor to the synthetic reaction is the length of the polysaccharide chain, or branch, produced. It was found impossible to build up a synthetic chain or branch exceeding 200 glucose units. Indeed, it was found that if the initial ratio of concentrations of glucose-1-phosphate to terminal glucose units of glycogen was greater than 200, the reaction failed to reach equilibrium. The result was also substantially the same when amylopectin and when amylose preparations were used as activators. Estimations of chain lengths produced were made from the amount, or number of glucose-1-phosphate units which had reacted and the number of terminal groups known to have been supplied as nuclei to initiate the reaction. The result was confirmed by an isolation of the synthetic material and an estimation of chain lengths by methylation end group analysis. Synthesis of a material from glucose-1-phosphate and potato phosphorylase which had the properties of an amylose was reported by Haworth *et al.* (205) and the average chain length was found to be about 90 glucose residues by methylation end group analysis. This value was found to be consistent with viscosity, copper reducing value, and, subsequently, osmotic pressure measurements on the methylated product, in that all measurements indicated chain lengths somewhat under 200 glucose residues.

The reason that longer linear lengths cannot be produced is not understood at the present time. However, two important conclusions relating to starch structure follow. Terminal branches on branched structures may be as long as 200 glucose units, but not longer, and these, when they form, may approach the linear molecules in some of their properties, such as their tendency to retrograde and to stain blue with iodine (206-208). It also follows that unbranched amylose chains do not exceed a length of about 200 glucose residues, or about the order of magnitude of the fraction readily extractable from corn starch granules by solution in water at 70°C. Longer amylose molecules very likely contain one, or more anomalous linkages which originate either by way of a part of the branched nuclei which primed the reaction and which has become a part of the synthetic molecule, or by another mechanism which will now be discussed.*

Although it was recognized in early work that both muscle and potato phosphorylases synthesized a blue, iodine-stained amylose molecule, it was also noted by the Coris and their coworkers that the enzyme prepared from other sources such as liver and yeast formed a product which resembled glycogen (or amylopectin) which gave a brown, or purplish-brown color with iodine and is now known to be a highly branched material. Subsequent studies by these workers have shown that both liver and heart contain a heat-labile protein fraction which, by itself is inactive in the presence of glucose-1-phosphate. However, when crystalline muscle phosphorylase is added to this mixture there is a pronounced induction period followed by the production of glycogen, or amylopectin like material at a rate which is strongly indicative of an autocatalyzed reaction (203).

---

*The apparent explanation that the reaction is halted for physical reasons in that as the chain length approaches 200 units the product retrogrades, or becomes insoluble, is not applicable to the experiment (203) wherein dilute, stable solutions of amylose were incubated with phosphorylase over extended periods of time and it was found that the chain length could not be increased more than 20 glucose units. See also experiments in ref. 206 using solutions of synthetic muscle and potato polysaccharides.

It would be expected, of course, in this case, that the creation of a multi-branched product would activate the primary phosphorylase reaction by providing an increasingly large number of terminal groups to act as nuclei for the production of new linear members, and as the linear members grew, both in length and in number, the opportunity for the coenzyme to function would likewise be increased.

From these experiments it is apparent that the formation of a branched type of starch molecule is due to the simultaneous action of two enzymic factors (or an enzyme and co-enzyme), the first being the enzyme phosphorylase which by itself creates specifically 1-4, α-glucosidic linkages and the second being a factor which by itself is inactive, but which in the presence of phosphorylase can produce the anomalous, 1-6, α-glucosidic linkage. Depending on the ratio of the two, a variety of branched type of polysaccharides could be formed such as glycogen or amylopectins, each differing somewhat in structure.

From a further study of potato juice, Haworth *et al.* (209) and Bourne and Peat (210) have reported the separation of a branching factor which they have called the Q-enzyme. This, by itself, is inactive with glucose-1-phosphate but in the presence of phosphorylase synthesizes an amylopectin like material. The synthetic amylopectin was found to be non-reducing, non-retrograding, converted by beta-amylase to the extent of 46%, and stained red-purple with iodine. Methylation and end-group assay of the methyl ether showed unit chains of 20 glucose units. The course of the synthesis was given as follows. Phosphorylase produces from glucose-1-phosphate, unit chains of some 20 glucose residues, called pseudo-amylose, containing only 1-4, α-glucosidic linkages. These are then joined by the Q-enzyme through 1-6, α-glucosidic linkages to form a laminated structure which has been discussed previously. Bourne *et al.* (211) found that the Q-enzyme is able to convert amylose into an amylopectin type of molecule which stains red with iodine. The action of the Q-enzyme in this case is thought to be twofold. The amylose is first split into short, unbranched chains of pseudo-amylose from which amylopectin is synthesized by a lateral combination.

Considerable information exists, from which an outline for the ultimate synthesis of starch may be given. Glucose-1-phosphate (Cori ester (212)) can originate in living cells from several sources. Glucose may be phosphorylated directly to glucose-6-phosphate in the presence of adenosine triphosphate and the enzyme hexokinase (213-215). The adenosine triphosphate acts as a phosphate donor and supplies the energy required for this synthesis. Glucose-6-phosphate is transformed in a reversible reaction into glucose-1-phosphate by the enzyme phosphoglucomutase in the presence of Mg-ions (216-218). Glucose may develop in plant cells by photosynthesis.

Glucose-1-phosphate may also originate by way of glucose-6-phosphate from fructose. Fructose-6-phosphate is transformed into the corresponding glucose phosphate in a reversible reaction by means of isomerase, an enzyme shown to exist in muscle and other tissue by Lohmann (219) and in plant cells by Tanko (220). The fructose phosphate may be an intermediate in the synthesis of glucose from such carbohydrate precursors (or degradation products) as pyruvic, lactic, fumaric, and malic acids or glycerol. James *et al.* (221) observed that when a hexosediphosphate is added to barley sap it is decomposed to pyruvic acid and when crude pea extracts are allowed to act on starch in the presence of inorganic phosphate, fructose-1-6-diphosphate (Hardin and Young ester) may be isolated. The latter may be interpreted to indicate that an enzyme which transforms fructose-6-phosphate into fructose-1-6-diphosphate and which is similar to that found in yeast and animal tissues, also exists in higher plants, and the former observation to indicate that pyruvic acid is a normal intermediate in the respiration of plants. The formation of phosphopyruvic acid from malate has been observed by Kalckar (214) and from lactate, in the presence of fluoride, by Ferdman and Epstein (222). Colowick *et al.* (215) concluded that the synthesis of glucose takes place by way of phosphopyruvic acid and showed that when kidney slices are held anerobically with pyruvate, glucose is produced. In a recent study, Calvin and Benson (223) have found considerable support for this general theory and have supplied several of the steps which were lacking in our knowledge of photosynthesis. The path of carbon was traced by exposing green algae to $C^{14}O_2$. Under certain conditions a triose phosphate and phosphoglyceric acid, both containing radioactive carbon, were isolated. From these and other significant data on the order in which C^{14} appeared in intermediates it was concluded that acetate is converted by a carboxylation and reduction reaction through pyruvate to phosphoglyceric acid and thence to a triose-3-phosphate.

Accordingly therefore, the ultimate synthesis of starch proceeds by a very involved and extended series of reactions, the essentials of which may be indicated as follows:

$$\text{Acetate} \xrightarrow[\text{H}]{\text{CO}_2} \text{Pyruvate} \overset{\text{PO}_4}{\rightleftharpoons} \text{Phosphoglyceric acid} \rightleftharpoons \text{Triose-3-phosphate}$$
$$\rightleftharpoons \text{Fructose-1-6-diphosphate} \rightleftharpoons \text{Fructose-6-phosphate} \rightleftharpoons$$
$$\text{Glucose-6-phosphate} \rightleftharpoons \text{Glucose-1-phosphate} \rightleftharpoons \text{Starch.}$$

Inasmuch as hexose-6-phosphates may be de-phosphated by the enzyme phosphatase, which is widely distributed in nature, but not the aldohexose-1-phosphates (224), fructose-6-phosphate may give rise to fructose. Hence, the possibility of this sugar uniting with glucose-1-phosphate to form sucrose at intermediate stages in plant respiration, or in carbohydrate synthesis, is evident, particularly in view of the work of Hassid and coworkers (225) and of Kagan *et al.* (226) and a biochemical relationship between sucrose, so commonly found in plants, and starch is provided.

Meagher and Hassid (227) have shown that maltose-1-phosphate is not converted into polysaccharides by phosphorylase.

6. Conclusions. In summation it may be said that experiments on the fractionation of starch into its constituent polymers, a study of the physico-chemical properties of these fractions and of chemical reactions leading to their derivitization or degradation into molecules of known structure, and studies on the synthesis of the two major types of starch molecules, all give a great preponderance of evidence to support the view given in the introduction to this discussion on the chemical nature of starch.

As mentioned earlier, no one particular study, perhaps, affords a rigorous exposition as to the composition of starch and the structure of its components. However, the great mass of diverse data supplied by a large number of workers has provided a very satisfying concept which, it is believed, is now held by a large majority of those concerned with this phase of biological chemistry.

BIBLIOGRAPHY

1. Hurd, C. D., and Cantor, S. M., *J. Am. Chem. Soc.*, **60**, 2677 (1938).
2. Hurd, C. D., Liggett, R. W., and Gordon, K. M., *J. Am. Chem. Soc.*, **63**, 2656 (1941).
3. Karrer, P., *Helv. Chim. Acta*, **4**, 994 (1921); **6**, 402 (1923).
4. Haworth, W. N., and Percival, E. G. V., *J. Chem. Soc.*, **1931**, 1342.
5. Irvine, J. C., in Comprehensive Survey of Starch Chemistry, ed. by R. P. Walton, New York, 16 (1928).
6. Haworth, W. N., Hirst, E. L., and Webb, J. I., *J. Chem. Soc.*, **1928**, 2681.
7. Schoch, T. J., *Advances in Carbohydrate Chem.*, **1**, 247 (1945).
8. Schoch, T. J., Starch and Its Derivatives, Ed. by J. A. Radley, 3rd Ed., London. (In press).

9. Gatin-Gruzewska, Z., *Compt. rend.*, **146**, 540 (1908); **152**, 785 (1911).
10. Samec, M., and Haerdtl, H., *Kolloid-Beihefte*, **12**, 281 (1920).
11. Samec, M., and Mayer, A., *Kolloid-Beihefte*, **13**, 272 (1921).
12. Samec, M., Kolloidchemie der Stärke, Dresden (1927).
13. Samec, M., and Blinc, M., *Kolloid-Beihefte*, **47**, 371 (1938).
14. Hess, K., and Krajnc, B., *Ber.*, **73**, 976 (1940).
15. Hess, K., and Steurer, E., **73**, 1076 (1940).
16. Maquenne, L., and Roux, E., *Compt. rend.*, **140**, 1303 (1905). See also *Bull. soc. chim.*, **33**, 723 (1905).
17. Tanret, C., *Compt. rend.*, **158**, 1353 (1914); **159**, 530 (1914).
18. Tanret, C., *Bull. soc. chim.*, **17**, 83 (1915).
19. Sherman, H. C., and Baker, J. C., *J. Am. Chem. Soc.*, **38**, 1885 (1916).
20. Baldwin, M. E., *J. Am. Chem. Soc.*, **52**, 2907 (1930).
21. Meyer, K. H., Bretano, W., and Bernfeld, P., *Helv. Chim. Acta*, **23**, 845 (1940).
22. Meyer, K. H., Bernfeld, P., and Wolff, E., *Helv. Chim. Acta*, **23**, 854 (1940).
23. Meyer, K. H., Wertheim, M., and Bernfeld, P., *Helv. Chim. Acta*, **23**, 865 (1940
24. Kerr, R. W. and Severson, G. M., *J. Am. Chem. Soc.*, **65**, 193 (1943).
25. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, 148 (1944).
26. Kerr, R. W. and Trubell, O. R., *Paper Trade J.*, (Tappi) **117**, 25 (1943).
27. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, 333 (1944).
28. Bates, F. L., French, D., and Rundle, R. E., *J. Am. Chem. Soc.*, **65**, 142 (1943).
29. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 558 (1943).
✓30. Schoch, T. J., and Jensen, C. C., *Ind. Eng. Chem.*, **12**, 531 (1940).
31. Kerr, R. W., and Cleveland, F. C. (In preparation).
32. Schoch, T. J., *Cereal Chem.*, **18**, 121 (1941).
33. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2957 (1942).
34. Wiegel, E., *Z. physik. Chem.*, **188**, 137 (1941).
35. Meyer, K. H., and Fuld, M., *Helv. Chim. Acta*, **24**, 1408 (1941).
36. Wiegel, E., *Kolloid. Z.*, **102**, 145 (1943).
37. Bear, R. S., *J. Am. Chem. Soc.*, **66**, 2122 (1944).
✓38. Whistler, R. L., and Hilbert, G. E., *J. Am. Chem. Soc.*, **67**, 1161 (1945).
✓39. Haworth, W. N., Peat, S., and Sagrott, P. E., *Nature*, **157**, 19 (1946).
40. Cleveland, F. C., and Kerr, R. W., *Cereal Chem.*, **25**, 133 (1948).
✓41. Kerr, R. W., *J. Am. Chem. Soc.*, **67**, 2268 (1945)
42. Cleveland, F. C., and Kerr, R. W., Osmotic Pressure Measurements on Corn Amylose (In press).
43. Kerr, R. W., *Arch. Biochem.*, **7**, 377 (1945).
44. Meyer, K. H., Noelting, G., and Bernfeld, P., *Experientia*, **3**, 370 (1947). See also *Helv. Chim. Acta*, **31**, 108 (1948).
45. McCready, R. M., and Hassid, W. Z., *J. Am. Chem. Soc.*, **65**, 1154 (1943).
✓46. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.*, **66**, 111 (1944)
47. Horan, F. E., Dissertation, Columbia University, New York (1944).
48. Higginbotham, R. S., and Morrison, G. A., Chemistry & Industry, **77** (1945)
49. Haworth, W. N., The Constitution of Sugars, London (1929).
50. Haworth, W. N., *Ann. Repts Chem. Soc.*, London, **102** (1931).
51. Hirst, E. L., Plant, M. M. T., and Wilkinson, M. D., *J. Chem. Soc.*, **1932**, 2375.
52. Haworth, W. N., Hirst, E. L., and Woolgar, M. D., *J. Chem. Soc.*, **1935**, 177.

53. Baird, K. D., Haworth, W. N., and Hirst, E. L., *J. Chem. Soc.*, **1935**, 1201.
54. Hassid, W. Z., and Dore, W. H., *J. Am. Chem. Soc.*, **59**, 1503 (1937).
55. Richardson, W. A., Higginbotham, R. S., and Farrow, F. D., *J. Textile Inst.*, **27**, 131 (1936).
56. Staudinger, H., *Naturwissenschaften,* **25**, 673 (1937).
57. Staudinger, H., and Husemann, E., *Ber.,* **70**, 1451 (1937).
58. Staudinger, H., and Husemann, E., *Ann.,* **527**, 195 (1937).
59. Bell, D. J., *Biochem. J.,* **30**, 1612 (1936).
60. Haworth, W. N., Hirst, E. L., and Isherwood, F. A., *J. Chem. Soc.*, **1937**, 577.
61. Carter, S. R., and Record, B. R., *J. Chem. Soc.,* **664** (1939).
62. Freudenberg, K., and Boppel, H., *Ber.,* **71**, 2505 (1938).
63. Freudenberg, K., Boppel, H., and Meyer-Delius, M., *Naturwissenschaften,* **26**, 123 (1938).
64. Freudenberg, K., and Boppel, H., *Ber.,* **609** (1940).
65. Freudenberg, K., *Naturwissenschaften,* **28**, 264 (1940).
66. Hirst, E. L., and Young, G. T., *J. Chem. Soc.,* **1247** (1938).
67. Barker, C. C., Hirst, E. L., and Young, G. T., *Nature,* **147**, 296 (1941).
68. Haworth, W. N., *Chemistry & Industry,* **58**, 917 (1939).
69. Haworth, W. N., Heath, R. L., and Peat, S., *J. Chem. Soc.,* **1942**, 55.
70. Hassid, W. Z., and McCready, R. M., *J. Am. Chem. Soc.,* **65**, 1157 (1943).
71. Hirst, E. L., and Young, G. T., *J. Chem. Soc.,* **1939**, 1471.
72. Hess, K., and Lung, K. H., *Ber.* **71**, 815 (1938).
73. Hess, K., and Lung, K. H., *Ber.* **70**, 1259 (1937).
74. Shorigina, N. N., Chem. Met. Eng., 53, 288 (1946). See also *Zhur. Obschei. Khim. NIV,* (*J. Gen. Chem. US.S.R.*) **7-8**, 825 (1944).
75. Hodge, J. E., Private communication.
76. Averill, F. J., and Peat, S., *J. Chem. Soc.,* **1938**, 1244.
77. MacDonald, J. Y., *J. Am. Chem. Soc.,* **57**, 771 (1935).
78. Bell, D. J., *Biochem. J.,* **29**, 2031 (1935).
79. Brown, F., and Jones, J. K. N., *J. Chem. Soc.,* **1947**, 1344.
80. Caldwell, C. G., and Hixon, R. M., *J. Am. Chem. Soc.,* **63**, 2876 (1941).
81. Levine, M., Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.,* **64**, 2331 (1941).
82. Neumann, F., and Hess, K., *Ber.,* **70**, 721 (1937).
83. Coleman, G. H., Rees, D. E., Sunberg, R. L., and McCloskey, C. M., *J. Am. Chem. Soc.,* **67**, 381 (1945).
84. Peat, S., and Whetstone, J., *J. Chem. Soc.,* **1940**, 276.
85. Myrbäck, K., and Tamm, *Svensk Kem. Tid.,* **53**, 441 (1941).
86. Mertzweiller, J. K., Carney, D. M., and Farley, F. F., *J. Am. Chem. Soc.,* **65**, 2367 (1943).
87. Hixon, R. M., Private communication.
88. Jones, J. K. N., *J. Chem. Soc.,* **1944**, 333.
89. Bell, D. J., *J. Chem. Soc.,* **1944**, 473.
90. Georges, L. W., Bower, R. S., and Wolfrom, M. L., *J. Am. Chem. Soc.,* **68**, 2169 (1946).
91. Pigman, W. W., *J. Research,* **33**, 105 (1944).
92. Haworth, W. N., Kitchen, H., and Peat, S., *J. Chem. Soc.,* **1943**, 619.
93. Balls, A. K., Thompson, R. R., and Walden, M. K., *J. Biol. Chem.,* **163**, 571 (1946).
94. Meyer, K. H., Fischer, E. H., and Bernfeld, P., *Helv. Chim. Acta,* **30**, 64 (1947); *Experientia,* **3**, 106 (1947).

95. Meyer, K., Fuld, M., and Bernfeld, P., *Experientia,* **3**, 411 (1947).
96. Myrbäck, K., *Chem. Zentr.,* II, 201 (1938).
97. Myrbäck, K., and Ahlborg, K., *Biochem. Z.,* **307**, 69 (1940).
98. Sillen, L. G., and Myrbäck, K., *Svensk Kem. Tid.,* **55**, 345 (1943).
99. Myrbäck, K., and Lundberg, B., *Svensk Kem. Tid.,* **55**, 36 (1943).
100. Myrbäck, K., and Thorsell, W., *Svensk Kem. Tid.,* **55**, 178 (1943).
101. Myrbäck, K., *Svensk Kem. Tid.,* **56**, 60 (1944); *The Svedberg (Mem. Vol.)* 508 (1944).
102. Myrbäck, K., and Martelius, B., *Biochem. Z.,* **316**, 414 (1944).
103. Myrbäck, K., and Ortenblad, B., and Thorsell, W., *Biochem. Z.,* **316**, 424 (1944).
104. Myrbäck, K., and Ortenblad, B., *Biochem. Z.,* **316**, 429 (1944).
105. Myrbäck, K., Stenlid, G., and Nycander, G., *Biochem. Z.,* **316**, 433 (1944).
106. Myrbäck, K., Ahlborg, K., and Ortenblad, B., *Biochem. Z.,* **316**, 444 (1944).
107. Myrbäck, K., and Sillen, L. G., *Svensk Kem. Tid.,* **56**, 60 (1944).
108. Sillen, L. G., and Myrbäck, K., *Svensk Kem. Tid.,* **56**, 142 (1944).
109. Myrbäck, K., *Svensk Kem. Tid.,* **58**, 119 (1946).
110. Myrbäck, K., and Lunden, R., *Arkiv Kemi, Mineral. Geol.,* **23**, 10 (1946).
111. Hanes, C. S., *New Phytologist,* **38**, 231 (1937).
112. Bernfeld, P., and Studer-Pecha, H., *Helv. Chim. Acta,* **30**, 1895 (1947).
113. Samec, M., and Battestin, M., *Z. physiol. Chem.,* **248**, 117 (1937).
114. Samec, M., and Waldsmidt-Leitz, E., *Z. physiol. Chem.,* **203**, 16 (1931).
115. Montgomery, E. M., Weakley, F. B., and Hilbert, G. E., *J. Am. Chem. Soc.,* **69**, 2249 (1947).
116. Georges, L. W., *J. Am. Chem. Soc.,* **69**, 473 (1947).
117. Kerr, R. W., and Schink, N. F., *Ind. Eng. Chem.,* **33**, 1418 (1941).
118. Kerr, R. W., Meisel, H., and Schink, N. F., *Ind. Eng. Chem.,* **34**, 1232 (1942).
119. Corman, J., and Langlykke, A. F., The Evaluation of Starch Hydrolyzing Mold Enzymes. Abstract of Papers, American Association of Cereal Chemists Annual Meeting, Kansas City, Mo., May 1947.
120. Meyer, K. H., and Bernfeld, P., *Helv. Chim. Acta,* **23**, 875 (1940).
121. Danielsson, C. E., *Nature,* **160**, 899 (1947).
122. Bernfeld, P., and Studer-Pecha, H., *Helv. Chim. Acta,* **30**, 1904 (1947).
123. Myrbäck, K., and Magnusson, B., *Arkiv Kemi, Mineral., Geol.,* **A20**, 22 (1945)
124. Bawn, C. E. H., Hirst, E. L., and Young, G. T., *Trans. Faraday Soc.,* **36**, 880 (1940).
125. Pacsu, E., and Hiller, L. A., Jr., *Textile Research J.,* **16**, 243 (1946).
126. Pacsu, E., *Textile Research J.,* **17**, 405 (1947).
127. Halsall, T. G., Hirst, E. L., and Jones, J. K. N., *Nature,* **159**, 97 (1947)
128. Naegeli, C. W., *Ann.,* **173**, 218 (1874).
129. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, 167 (1944).
130. Unruh, C. C., and Kenyon, W. D., *J. Am. Chem. Soc.,* **64**, 127 (1942)
131. Malaprade, L., *Bull. soc. chim.,* **43**, 683 (1928).
132. Karrer, P., and Pfaehler, K., *Helv. Chim. Acta,* **17**, 766 (1934)
133. Herrissey, H., Fleury, P., and Joly, M., *J. pharm. chim.,* **20**, 149 (1934)
134. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **58**, 378 (1936)
135. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **59**, 994 (1937).
136. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **59**, 2049 (1937).
137. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **60**, 989 (1938).

138. Caldwell, C. G., and Hixon, R. M., *J. Biol. Chem.*, **123**, 595 (1938).
139. Grangaard, D. H., Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.*, **61**, 1290 (1939).
140. Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.*, **64**, 585 (1942).
141. Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.*, **64**, 589 (1942).
142. Grangaard, D. H., Gladding, E. K., and Purves, C. B., *Paper Trade J.*, **115**, 41 (1942).
143. Brown, F., Dunstan, S., Halsall, T. G., Hirst, E. L., and Jones, J. K. N., *Nature*, **145**, 785 (1945).
144. Halsall, T. G., Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.*, **1947**, 1399.
145. Halsall, T. G., Hirst, E. L., Jones, J. K. N., and Roudier, A., *Nature*, **160**, 899 (1947).
146. Newton, J. M., Dissertation, Iowa State College, Ames (1941).
147. Richardson, W. A., Higginbotham, R. S., and Farrow, F. D., *J. Textile Inst.*, **27**, 131 (1936).
148. Rundle, R. E., and Baldwin, R. R., *J. Am. Chem. Soc.*, **65**, 554 (1943).
149. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 1707 (1943).
150. Rundle, R. E., and Edwards, F. C., *J. Am. Chem. Soc.*, **65**, 2200 (1943).
151. Rundle, R. E., Foster, J. F., and Baldwin, R. R., *J. Am. Chem. Soc.*, **66**, 2116 (1944).
152. Stein, R. S., and Rundle, R. E., *J. Chem. Phys.*, **16**, 195 (1948).
153. Dube, H. A., Dissertation, Iowa State College, Ames (1947).
154. Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.*, **66**, 557 (1944).
155. Huggins, M. L., *J. Am. Chem. Soc.*, **64**, 2716 (1942).
156. Huggins, M. L., *Ind. Eng. Chem.*, **35**, 980 (1943).
157. Speiser, R., and Whittenberger, R. T., *J. Chem. Phys.*, **13**, 349 (1945).
158. Bartovics, A., and Mark, H., *J. Am. Chem. Soc.*, **65**, 1901 (1943).
159. Lauffer, M. A., *Chem. Revs.*, **31**, 561 (1942).
160. Huggins, M. L., *J. Phys. Chem.*, **42**, 911 (1938); **43**, 439 (1939).
161. Dombrow, B. A., Dissertation, Columbia, New York (1944).
162. Simha, R., *J. Phys. Chem.*, **44**, 25 (1940).
163. Fox, T. G., Dissertation, Columbia University, New York (1943); also Fox, T. G., and Beckmann, C. O., Abstracts, American Chem. Soc. Meeting, Pittsburg (1943).
164. Edwards, D. W., and Ripperton, J. C., *J. Agr. Research*, **47**, 179 (1933).
165. Northrop, J. H., and Nelson, J. M., *J. Am. Chem. Soc.*, **38**, 472 (1916).
166. Sherman, H. C., and Baker, J. C., *J. Am. Chem. Soc.*, **38**, 1885 (1916).
167. Posternak, T., *Helv. Chim. Acta*, **18**, 1351 (1935).
168. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2954 (1942).
169. Briggs, D. R., and Hanig, M., *Cereal Chem.*, **23**, 277 (1946).
170. Sostegni, L., *Gazz. chim. ital. Palermo*, **15**, 376 (1885).
171. Taylor, T. C., and Nelson, J. M. *J. Am. Chem. Soc.*, **42**, 1726 (1920).
172. Taylor, T. C., and Lehrman, L., *J. Am. Chem. Soc.*, **48**, 1739 (1926).
173. Taylor, T. C., and Iddles, H. A., *Ind. Eng. Chem.*, **18**, 713 (1926).
174. Taylor, T. C., and Werntz, J. H., *J. Am. Chem. Soc.*, **49**, 1584 (1927).
175. Taylor, T. C., and Walton, R. P., *J. Am. Chem. Soc.*, **51**, 3431 (1929); see also, Walton, R. P., Dissertation, Columbia University, New York (1929).
176. Taylor, T. C., and Sherman, R. T., *J. Am. Chem. Soc.*, **55**, 258 (1933).
177. Lehrman, L., *J. Am. Chem. Soc.*, **54**, 2527 (1932).
178. Lehrman, L., and Kabat, E. A., *J. Am. Chem. Soc.*, **55**, 580 (1933).

179. Kerr, R. W., Trubell, O. R., and Severson, G. M., *Cereal Chem.*, **19**, 64 (1942).
180. Rask, O. S., and Phelps, J. K., *Ind. Eng. Chem.*, **17**, 187 (1925).
181. Schoch, T. J., *J. Am. Chem. Soc.*, **60**, 2824 (1938).
182. Lehrman, L., *J. Am. Chem. Soc.*, **64**, 2144 (1942).
183. Schoch, T. J., and Williams, C. B., *J. Am. Chem. Soc.*, **66**, 1232 (1944).
184. Mikus, F. F., Hixon, R. M., and Rundle, R. E., *J. Am. Chem. Soc.*, **68**. 1115 (1946).
185. Clayson, D. H. F., and Schryver, S. B., *Biochem. J.*, **17**, 493 (1923).
186. Schryver, S. B., and Thomas, E. M., *Biochem. J.*, **17**, 497 (1923).
187. Ling, A. R., and Nanji, D. R., *J. Chem. Soc.*, **1925**, 127.
188. Samec, M. Kolloidchemie der Stärke, Dresden, 36 (1927).
189. Kerr, R. W., and Trubell, O. R., *Cereal Chem.*, **18**, 530 (1941).
190. Samec, M., *Kolloid-Beihefte*, **40**, 449 (1934).
191. Bungenberg de Jong, H. G., *Biochem. Z.*, Series of papers (1929-1933).
192. Koets, P., *J. Phys. Chem.*, **40**, 1191 (1936).
193. Koets, P., *Proc. Koninke Akad. Wetenschap. Amsterdam*, **38**, 3 (1935).
194. von Przylecki, St. J., and Majmin, R., *Biochem. Z.*, **277**, 420 (1935).
195. Dobry, A., *Compt. rend.*, **222**, 693 (1946).
196. Cori, C. F., and Cori, G. T., *Ann. Rev. Biochem.*, **10**, 151 (1941).
197. Hanes, C. S., Nature, **145**, 348 (1940).
198. Hanes, C. S., *Proc. Roy. Soc. London*, **B128**, 421 (1940).
199. Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, **131**, 397 (1939).
200. Cori, C. F., and Cori, G. T., *J. Biol. Chem.*, **135**, 793 (1940).
201. Hanes, C. S., *Proc. Roy. Soc. London*, **B129**, 174 (1940).
202. Hanes, C. S., and Maskell, E. J., *Biochem. J.*, **36**, 76 (1942).
203. Cori, G. T., Swanson, M. A., and Cori, C. F., *Federation Proc.*, **4**, 234 (1945).
204. Hidy, P. H., and Day, H. G., *J. Biol. Chem.*, **152**, 479 (1944).
205. Haworth, W. N., Heath, R. L., and Peat, S., *J. Chem. Soc.*, **1942**, 55.
206. Hassid, W. Z., Cori, G. T., and McCready, R. M., *J. Biol. Chem.*, **148**, 89 (1943).
207. Cori, C. F., Schmidt, G., and Cori, G. T., *Science*, **89**, 464 (1939).
208. Bear, R. S., and Cori, C. F., *J. Biol. Chem.*, **140**, 111 (1941).
209. Haworth, W. N., Peat, S., and Bourne, E. J., *Nature*, **154**, 236 (1944).
210. Bourne, E. J., and Peat, S., *J. Chem. Soc.*, **1945**, 877.
211. Bourne, E. J., Macey, A., and Peat, S., *J. Chem. Soc.*, **1945**, 882.
212. Cori, C. F., and Cori, G. T., *Proc. Soc. Exptl. Biol. Med.*, **34**, 702 (1936).
213. Meyerhof, O., *Biochem. Z.*, **183**, 176 (1927).
214. Kalckar, H., *Enzymologia*, **2**, 47 (1937).
215. Colowick, S. P., Welch, M. S., and Cori, C. F., *J. Biol. Chem.*, **133**, 359 (1940)
216. Cori, G. T., Colowick, S. P., and Cori, C. F., *J. Biol. Chem.*, **124**, 543 (1938).
217. Sutherland, E. W., Colowick, S. P., and Cori, C. F., *J. Biol. Chem.*, **140**, 309 (1941).
218. Colowick, S. P., and Sutherland, E. W., *J. Biol. Chem.*, **144**, 423 (1942).
219. Lohmann, K., *Biochem. Z.*, **262**, 137 (1933).
220. Tanko, B., *Biochem. J.*, **30**, 692 (1936).
221. James, W. O., James, G. M., and Bunting, A. H., *Biochem. J.*, **35**, 588 (1941)
222. Ferdman, D. L., and Epstein, S. F., *Science*, **91**, 365 (1940).
223. Calvin, M., and Benson, A. A., *Science*, **107**, 476 (1948).
224. Hassid, W. Z., *Quart. Rev. Biol.*, **18**, 311 (1943).
225. Doudoroff, M., Kaplan, N., and Hassid, W. Z., *J. Biol. Chem.*, **148**, 67 (1943)
226. Kagan, B. O., Latker, S. N., and Zfasman, E. M., *Biokhimiya*, **7**, 92 (1942)
227. Meagher, W. R., and Hassid, W. Z., *J. Am. Chem. Soc.*, **68**, 2135 (1946)

SECTION IV. REACTIONS

REACTIONS

From a discussion in previous chapters of the composition of starch and of the general configuration of its component molecules, it is apparent that starch may enter into several types of reactions. Being composed of many glucose units united by glucosidic linkages, the molecules may be hydrolyzed by acids at any or all of the points where a glucoside bond occurs. Conversely, condensation may result wherever the molecule ends in an aldehyde group or wherever there is a free, reactive hydroxyl group. The condensation may be with a hydroxyl group on the same or different carbohydrate chain that contains the aldehyde group, or it may be with the terminal aldehyde and an alcoholic hydroxyl group of a non-carbohydrate substance. Or again, the condensation of aldehyde and hydroxyl may be from a non-carbohydrate carbonyl group to a carbohydrate hydroxyl group. The latter type of reaction is discussed below under "Miscellaneous reactions." Some of the other types are treated in chapters, "Acid hydrolysis of starch" and "Dextrinization." The hydrolysis of starch, and possibly condensations to some extent, may be induced in the presence of rather specific, organic catalysts which are called enzymes. Two chapters are included which deal with starch-splitting enzymes and their modification of starch.

Since the carbohydrate molecule contains alcoholic hydroxyl groups of both the primary and secondary type and at least potential aldehyde groups, the molecule may be oxidized at one or many of these points; the hydroxyl groups are changed to carbonyl groups and the carbonyl groups to carboxyl groups. The ketone carbonyl groups from the secondary alcoholic hydroxyl groups are oxidized to acids with a splitting of carbon to carbon valences. These reactions are discussed in the chapter, "Oxidation of starch." Aldehyde groups and the aldehydic carbonyl groups which result when glucosidic linkages rupture may be reduced to alcoholic groups. A section, "Hydrogenation of starch," is included.

Alcoholic hydroxyl groups may be derivatized to esters, both with organic and inorganic acids, preferably by the use of acid intermediates such as acid chlorides and anhydrides. The hydroxyl groups of starch may be converted to ethers and to alcoholates with metals or their hydroxides. The chapter, "Derivatives of Starch," is devoted to a discussion of these types of reactions.

Starch enters into several other reactions; *e.g.,* when starch is treated with hot sodium hydroxide, it is progressively degraded into bodies of low molecular weight such as lactic acid. Starch is also degraded into bodies of low molecular weight by the extensive action of certain oxidizing agents, by very complicated enzyme systems, or by the effect of heat. The latter type of degradation may involve atmospheric oxidation, dehydration, or a combination of the two.

Some of the large scale manufacturing processes are based on several of the many reactions listed. These types in particular will be discussed together with a few other reactions of either technical importance or academic interest.

Some high polymers, such as starch, do not always react in a straightforward or predictable manner, as do bodies of low molecular weight, because they contain molecules that are so oriented, or cross-bonded, that the resultant forces tend to direct the point of attack of the reagent in respect to the many functional groups or linkages in the molecules. The crystallite structure, the extent to which it is disorganized, and the extent to which reassociations take place may exert a pronounced effect on the mode of chemical reactions involving starch. As an aid to a better understanding of starch reactions, this section is prefixed by a chapter on the hydrogen bond in starch.

CHAPTER IX

THE HYDROGEN BOND IN STARCH AS A BASIS FOR INTERPRETING ITS BEHAVIOR AND REACTIVITY

BY G. V. CAESAR

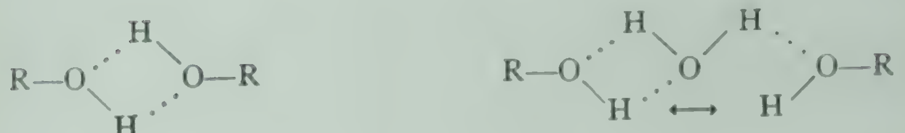
1. **Theory of the H Bond.** "It has been recognized in recent years that under certain conditions an atom of H is attracted by rather strong forces to two atoms, instead of only one, so that it may be considered to be acting as a bond between them. This is called the 'hydrogen bond' . . . It is now recognized that . . . the hydrogen bond is largely ionic in character and is formed only between the most electronegative atoms."

Pauling (1) so characterizes one of the most interesting, and perhaps the most important and far reaching, discoveries of modern physical chemistry. It is a fact now well established by experiments that H can act as a bond between the strongly electronegative atoms, F, O, and N, and, to a lesser extent under suitable conditions, between the weaker electronegative atoms, Cl and C, the link being expressed as $F-H\cdots F$, $O-H\cdots O$, $N-H\cdots N$, $N-H\cdots O$, $O-H\cdots Cl$, $C-H\cdots N$, etc., the dotted link representing the H bond in proton migration. For a more detailed discussion of fundamentals, the reader is referred to the work of Pauling (1). This brief treatise will be confined primarily to the rôle of the H bond in the physical chemistry of starch, with occasional reference to its rôle in the related subjects of cellulose and proteins.

2. **Association in Starch.** Association of hydroxylated compounds through hydrogen bonding is now well established through various physical methods, among which, spectroscopic studies in the infrared region are very important (1). Experimental difficulties have prevented the direct application of these methods to polysaccharides such as starch and cellulose but indirect evidence of such association is so convincing that hydrogen bonding is now generally accepted and its importance appreciated, not only in starch, but in cellulose chemistry (2). In the field of starch chemistry, T. C. Taylor, of Columbia University, was probably among the first to recognize its important role (3). It was his opinion (4) that the study of starch chemistry is incomprehensible without the postulation of micellar association through hydrogen bonding.

An attempt will be made to follow the behavior of starch in aqueous dispersions, its most characteristic and commercially useful state, and to postulate the association of primary valence chains through the H bond.

In formula (I) the association of 2 molecules of starch in the absence of



(I) (II)

Association of starch (schematic). The H bonds are represented by dots. (I) inactive form; (II) active form.

water and (II) the association through water is indicated schematically. Association (I) is of the type now recognized for many carboxylic acids. In a polyhydroxylated compound such as starch, the arrangement would obviously be expected to reduce the activity of the hydroxyl groups. Let us consider what happens to starch that is drastically desiccated or thoroughly retrograded. It becomes relatively indispersible (or insoluble) in water. In drastic drying there is evidence that more water is lost than appears to be naturally present, and in retrogradation there is also evidence that intramolecular water is eliminated. In both of these processes we might expect the internal arrangement to approach an association of type (I) in which neighboring OH groups directly associate or are bonded with one another, with consequent reduction in reactivity, or *availability*, of OH groups. In type (II), which may be designated as starch in a normal state, neighboring OH groups (the majority of which may possibly be represented by the primary alcohol groups at carbon atom 6, for reasons discussed elsewhere (5)) are linked through one or more molecules of H_2O which pry them apart and render them more reactive.* Starch normally contains a relatively large amount of available moisture, 10% or more, plus moisture which is emitted only under extreme conditions. On the assumption of association through hydrogen bonding, we should expect the easily expelled water to be loosely bonded to the exterior of the micelles and the balance to be bonded more or less intramicellarly as in (II).†

3. Hydration, Gelatinization, and Gelation in Terms of Changes in H Bonding. Now what may be the mechanism of hydration or gelatinization of normal starch? The hydration or gelatinization of starch is characterized, initially, by swelling of the granular packages, subsequently, by rupture and dispersion of these swollen packages, the degree of rupture and dispersion varying enormously and nearly always exhibiting marked heterogeneity.

Considering the characteristics of the phenomenon of the swelling of starch in aqueous media, it is noted, first of all, that it is occasioned by heating the water or by dissolving certain substances in a cold water suspension of starch. Starch swells slowly and only to a very limited extent in cold water. The probable mechanism which explains the initial hydration of starch is suggested in a recent contribution by Buswell *et al,* (6) on the effect of ions on the coordinated structure of water. The association of water (through H bonds) was studied by means of infrared absorption. From the results reported, the indications are that the coordinated structure of water is at least in part destroyed by 4 M concentrations of salts of the lyotropic series, NaI, NaSCN, NaBr, etc., the effect of the NaI being $>$ NaSCN $>$ NaBr. NaI and NaSCN, among certain other salts, and the caustic alkalies are known to be more or less effective starch-swelling agents in aqueous media. If these agents act essentially to destroy the association of water molecules, that is to reduce the aggregates of water molecules to smaller units approaching in dimensions single molecules of H_2O, it would be expected that the penetrating power of water would be increased, and consequently its ability to become *available* to the tightly packed aggregates of starch molecules would be increased. $O — H \cdots O$ bonds are known to be broken also by heating; hot water is less associated than cold water (7). The conclusion seems probable, therefore, that gelatinization (hydration) of starch is initiated by the more active, dissociated water molecules. It is also quite possible that the same agents which weaken the H bonds between the water molecules also tend to weaken the H bonds through which the starch molecules are oriented in the original granule.

---

*The O—H . . . O distance is 2.76A. (1).

†The fact that cellulose contains less moisture than starch may be due to its very different molecular form (5).

The swelling of the average granule, once started, proceeds with very great rapidity.‡ In Figures 68 and 69 we may follow the record of slow, uniform heating and cooling, respectively, of 20% concentrations of a normal corn starch, and of the same material when defatted with dioxane, according to Schoch (8). The assumption of hydrogen bonding clarifies this interesting record. In Fig. 68* the normal starch begins to be hydrated appreciably at about 152°F. and then swells so rapidly that a consistency value of 9 (9 watts) is reached at about 154.5°. From this point on the percentage rate of increase in consistency begins to decrease rapidly, attaining a peak consistency in the neighborhood of 165°. The swollen granules begin to rupture, under the influence of increasing heat and strong agitation, and spill out clumps of associated complexes which show a wide variation in size, shape, and degree of hydration. This process continues, in addition to the continuation in the swelling process of the more resistant granular packages, through the temperature range of approximately 165-195°F. In the neighborhood of 190-195° a third phase in the gelatinization begins, in which the paste body decreases rapidly as the water temperature approaches the boiling point. Microscopically this phase is characterized by a visual disappearance of organized structure. The latter has become so disintegrated and hydrated that its index of refraction approaches that of water. Actually, the structure is still highly and complexly organized, but it is in a heterogenous colloidal stage of micellar organization. Further reference will be made to these observations in a discussion of the kinematic viscosity tests. Hydrogen bonds are being continuously broken and reformed throughout the heating period indicated for the consistency curve, the dissociating water molecules force their way into the micellar jungle of bristling, hydroxyl groups which entrap and bind them, as in formula (II), free water is consumed or "bound," and the paste viscosity increased until the peak of the curve is reached. The energy supplied by increasing temperature and the internal shear (from agitation) then begin to destroy the $O - H \cdots O$ links holding the bound water molecules to the starch hydroxyls, and the viscosity rapidly decreases. Prolonged heating also causes more or less depolymerization, an effect which naturally promotes further dissociation, and the loosening of hydrogen-bonded water. Prolonged treatments, by boiling, to decrease paste viscosity in certain commercial applications of starch, however, may cause considerable degeneration of main valence polymers as well.

---

‡A small proportion of the smaller granules (by weight, not necessarily by number) may be relatively quite resistant to hydration and swell slowly. The writer has recently observed that in nearly all starches there appear to exist large numbers of almost infinitesimal granules which are relatively inert, even in strong reagents. Their nature is still unknown but it is suspected that they may play an important rôle in the behavior of the starch.

*Figs. 68 and 69 are plotted on semilog paper the better to represent, graphically, changes in all values for paste consistency on a scale which more correctly evaluates the magnitude of such changes.

The defatted starch in Fig. 68 shows several interesting departures from the curve of the untreated starch: gelatinization begins nearly 3° earlier and is well under way before the untreated starch begins to be hydrated; it attains a definitely lower peak, and the decrease in consistency takes place at a more constant rate, showing only a slight indication of the characteristic inflection or shoulder at 190-195°. This behavior is in full accord with what might be expected from assumptions based on the concept of hydrogen bonding: the fatty acids in starch would be attached or spread upon the OH groups by their polar "heads"* in the manner that fatty acids are known to spread upon water; this would tend to impede hydration. It might be anticipated, therefore, that defatted starch would gelatinize earlier and also break down or dissociate more uniformly.

---

*Schoch (8) shows that the fat is adsorbed on the starch.

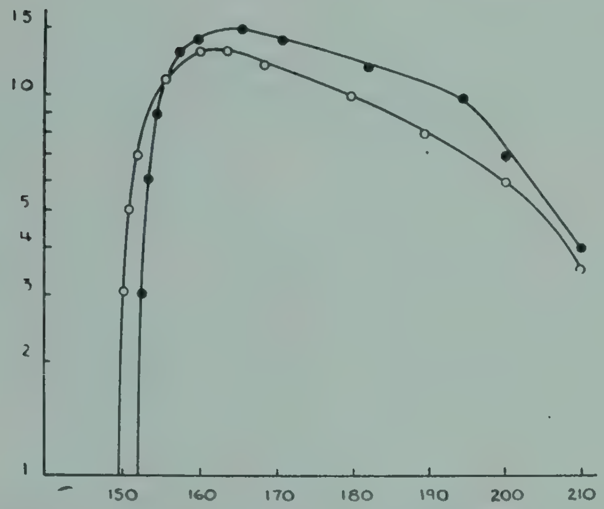


FIG. 68. Gelatinization record of normal and defatted corn starch in Stein Hall consistometer at 20% concentration in water. ● normal corn starch: ○ defatted corn starch. The abscissa, heating temperature in ° F.; the ordinate, consistency in watts.

Fig. 69 illustrates the thickening characteristics of these two starches after cooking to 210°F. The defatted starch congeals much faster, and below about 110°F. the curve flattens, indicating gel formation. The cooling curve of the untreated starch is more nearly linear, the slope is less, and the gelling tendency less pronounced. This is in accord with the well known paste-softening effect of fats, which interpose long fatty "tails," probably forcing apart the

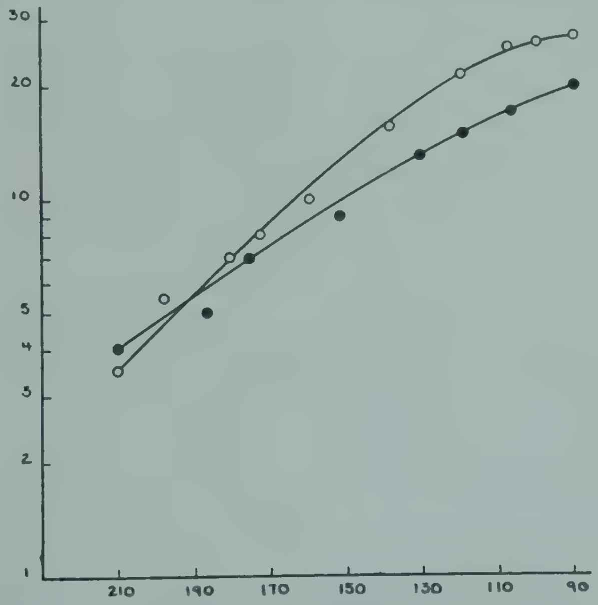


FIG. 69. Thickening record of normal and defatted corn starch in Stein Hall consistometer at 20% concentration in water. ● normal corn starch: ○ defatted corn starch. The abscissca, cooling temperature in ° F.; the ordinate, consistency in watts.

hydrated OH groups, thus promoting a looseness of micellar structure reflected in a softer, more plastic condition at moderate temperatures. In cooling the fat-free starch paste, the slowly reassociating complex of pure R — OH and H — O — H might be expected to become definitely less mobile due to forming a gel.

Thickening, or reassociation, is affected considerably by the rate of cooling, not only for aqueous starch dispersions (pastes) but for modified or degenerated starch dispersions. Rapid cooling increases the fluidity of such pastes: slow cooling promotes the formation of a gel, if such be structurally possible, the water being literally "bound" through a network of H bonds. A rapidly cooled paste is unstable, and tends to thicken progressively on standing, a common and usually unwelcome experience of starch technicians. The paste may be considered as being in a supercooled condition, the most stable hydrogen-bonded structure not having time to become fully developed. The thixotropy of starch pastes, *i.e.* their sensitivity to shearing forces, is well known. The weak, hydrogen-bonded, heterogeneous complex of *partially* disorganized starch and water might well be expected to be unstable to a marked degree. If, however, the starch structure be sufficiently disorganized and homogeneous, a well hydrated paste of such starch would be expected to be relatively stable, both to shearing forces, aging, and small changes in temperature. Its viscous properties would tend to show a similarity to the viscous properties of pure hydroxylated substances. It is known that prolonged mechanical agitation of starch pastes exerts a definite stabilizing effect.

4. Disorganized Starches. The above discussion is further illustrated by Fig. 70 in which the viscous properties in water dispersion of three polyhydroxylated substances, two starches in different states of disorganization and a sample of polyvinyl alcohol, are compared to pure water. From the fundamental equation for viscosity described by Eyring (9),

$$\eta = Be^{E_{vis}/RT}$$

the plot of the log of viscosity against $1/T$ should be linear, for non-associated liquids. E_{vis} is the "energy of activation for viscous flow." For associated liquids it is not independent of temperature and has a relatively high value, owing to the work required to break O — H ··· O bonds "before the activated state for flow can be attained . . . As the temperature is raised, there is a decrease in the number of hydrogen bonds that have to be broken before flow can occur, and hence the activation energy decreases . . . For associated liquids . . . viscosities are very much higher than for analogous non-associated substances, and the values decrease rapidly with increasing temperature. The plot of log η against $1/T$ is not linear" (7).

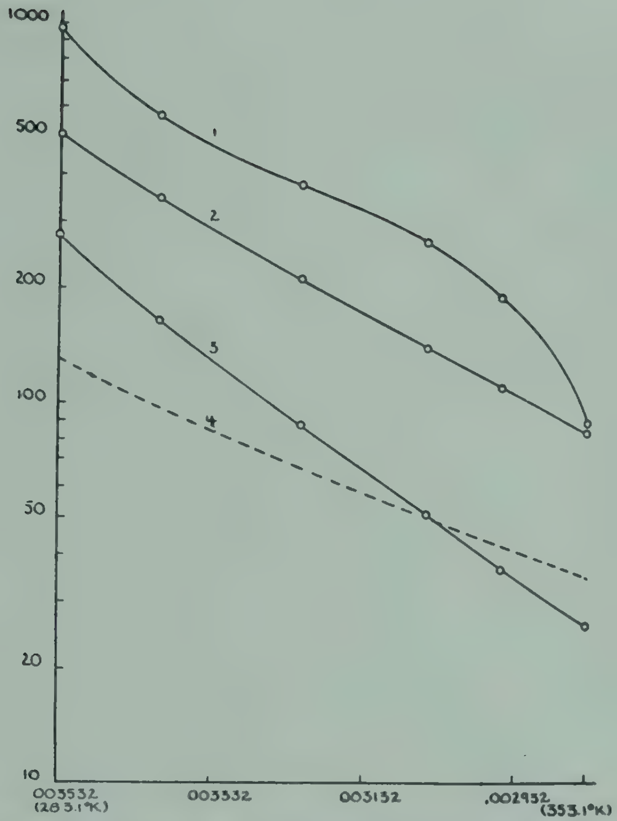


FIG. 70. Logarithmic scale for viscosity in centistokes (ordinate) *versus* $1/T$ (abscissa) for pastes and water. Curve 1, partially disorganized tapioca paste, 5%; Curve 2, highly disorganized tapioca paste, 10%; Curve 3, polyvinyl alcohol, 10%; Curve 4, water, viscosity scale 1/100.

In the study of the viscous properties represented in Fig. 70 the log of the kinematic viscosity was therefore plotted against $1/T$, over the range 283.1-353.1°K. (10-80°C).* In Fig. 70, Curve 1 represents an aqueous dispersion of a 5% concentration† of a high grade tapioca partially disorganized by thorough cooking and rapid agitation in a double action type of commercial mixer. Technically, it would be considered a well dispersed homogeneous fluid paste. Curve 2 represents the same starch at 10% concentration in an exceptional state of disorganization produced by a new process without chemical modification or appreciable degeneration of main valence polymers. Curve 3 represents a sample of polyvinyl alcohol of medium viscosity at 10% concentration. Curve 4 represents, for comparative purposes, the viscosity-temperature relationship of pure water (from data by Bingham), the viscosity scale being 1/100 of the scale for the starches and polyvinyl alcohol. Viscosity values were obtained in accurately calibrated Ostwald viscosimeters of the modified type used by the Socony Vacuum Laboratories, temperatures being controlled to ±0.05°F.

---

*I have found this method of plotting to be very informative and have generally adopted it for viscosity-temperature relationships.

†Kinematic viscosities over a wide temperature range could not be determined at 10%.

It is sufficiently apparent, at first glance, that the structure of the tapioca paste represented by Curve 1 is not homogeneous. Its percentage rate of increase below 80°C. is very great, then decreases, and finally increases again below about 20°C. The structure is a micellar conglomerate, its heterogeneity being strikingly revealed by a plotting of the log of viscosity against 1/T. Unfortunately, this is the type of starch dispersion too often used as the basis of much laborious research on "amylose" composition.

A very remarkable difference is shown in Curve 2. Obviously, this starch paste approaches complete homogeneity and an ideal solution. The plot of the log of viscosity against 1/T is remarkably flat, although still by no means linear, and the slope is considerable. Both of these results are strongly confirmative of hydrogen bonding (7).

Curve 3 is what might be expected from a homogeneous long chain hydroxylated polymer of the probable spatial structure of polyvinal alcohol. It shows more deviation from linearity than Curve 2, and the slope is definitely steeper. Tentatively this might be interpreted to signify the polyvinyl alcohol is more completely and strongly hydrogen-bonded than the disorganized starch (Curve 2).

The curve for water is curiously similar to Curves 2 and 3. Its slope is the least, as might be expected.

5. Location of H Bond in Starch and Influence of Position on Behavior. The presence of hydrogen bonds in starch is yet to be conclusively proved. By present experimental technique direct proof may well be impracticable. Indirect evidence is accumulating, however. In this connection the recent work of Mullen and Pacsu (10) should be noted. The heat of gelatinization of a number of starches has been studied in water and water-pyridine mixtures. The results indicate that these heats of gelatinization are of the order of magnitude to correspond to the energy required for one to two hydrogen bonds per glucose unit in starch. The theory of H bonds in starch is in sufficient accord with observed phenomena that even now little doubt should remain that starch, like other hydroxylated compounds, is more or less associated through O — H···O linkages. Probably, starch molecules are always more or less associated. This probability should be taken into account in all chemical and physical studies of starch in its many complex manifestations. Unbranched chains, branched chains at the 1-6 linkage, tightly packed or compressed coils or helices (5), extended or strained helices and the various combinations and proportions of such variables all would affect $O—H\cdots O$ linkages and the physical and chemical properties resulting from such manifold and complex association.

The precise location of the hydrogen bonding in starch molecules is still highly speculative. It seems reasonable to suppose that this location, primary hydroxyls as at carbon atom 6, or secondary hydroxyls as at carbon atoms 2 and 3, depends mostly upon spatial configuration involving the direction of the hydrogen atom, and the oxygen-oxygen distances dependent upon the configuration. This view is repeatedly stressed by Pauling (1) in the discussion of weak and strong H bonds, and it seems particularly plausible when molecules are constructed from models made to scale (5). It is probable, as has already been suggested, that the primary alcohol groups at carbon atom 6 form the strongest $O—H\cdots O$ bonds,* primarily for the reason that the spatial configuration of the unbranched chain should make them the strongest, an effect particularly potent in promoting retrogradation of unbranched chains. In branched chains, the "wedge" of glucopyranose inserted at the 1-6 position should, on spatial considerations alone, tend to inhibit retrogradation. The fact that unbranched chains show strong retrograding tendencies and branched chains do not is evidence for the configuration hypothesis in respect to the location of hydrogen bonding and, incidently, for the accuracy of the "picture" projected by recent models constructed. It should be reemphasized that the mechanism of starch association is probably through water molecules (except when the starch is retrograded or drastically dried). In the hydration or the peptization of starch, the thickness of the bound water layers and the uniformity of hydration may vary greatly, depending upon the state of aggregation of the unbranched and branched chains. The secondary OH groups should generally be more occluded than the protruding primary groups, especially in unbranched or so called "linear" chains. In branched chain aggregates, the secondary hydroxyl groups (for spatial reasons) should be more available to attract and build up more relatively stable starch-water complexes. Apparently they are more available and the starch-water complexes more stable (11).

The theory of hydrogen bonding supplies a means for clarifying many incongruities which appear to exist when the chemical structure of the starches is compared with the record of its varied behavior. It is hoped that this incomplete and necessarily speculative treatise may stimulate more use of this recent product of modern physical chemistry to rationalize the results of future research on the chemical and physical properties of the starches.

---

*This view is supported by the greater associative tendencies of primary alcohol groups when compared to secondary groups (1) although, as suggested in the text, the association is strongest for the hydroxyl groups of water molecules (Editor).

BIBLIOGRAPHY

1. Pauling, L., Nature of the Chemical Bond, Cornell Univ. Press, Ithaca, Chapter IX (1940).
2. Emil Ott, High Polymers, Interscience Publishers, New York, Volume 5 (1943).
3. Taylor, T. C., and Keresztesy, J. C., *Ind. Eng. Chem.*, **25**, 502 (1936).
4. Taylor, T. C., *private communications.*
5. Caesar, G. V., and Cushing, M. L., *J. Phys. Chem.*, **45**, 776 (1941).
6. Buswell, A. M., Gore, R. C., and Rodebush, W. H., *J. Phys. Chem.*, **45**, 543 (1941).
7. Glasstone, S., Laidler, K. J., and Eyring, H., Theory of Rate Processes, McGraw-Hill Book Co., New York, 505 (1941).
8. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2954 (1942).
9. Glasstone, S., Laidler, K. J., and Eyring, M., Theory of Rate Processes, McGraw-Hill Book Co., New York, 484 (1941).
10. Mullen, J. W., and Pacsu, E., *Ind. Eng. Chem.*, **34**, 807 (1942).
11. Kerr, R. W., *Paper Trade J.*, **115**, no. 22, 30 (1942).

Chapter X

DERIVATIVES OF STARCH

By Ed. F. Degering

1. Introduction.

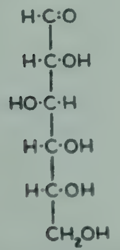
a. Definitions. The expression derivatives of starch, as used in this chapter, refers to compounds or mixtures which retain the intact starch molecule to an appreciable extent. The more important of these, according to studies to date, are the acyl, nitro, and alkyl derivatives. These are also referred to in the literature as starch alkanoates, as starch nitrates, and as starch ethers. The latter terms imply a chemical entity, whereas the former terms allow of greater freedom in defining the product. The author, accordingly, prefers to use the terms acyl, nitro, and alkyl derivatives of starch.

Whereas the definition in the preceding paragraph implies that the starch molecule tends to remain intact in the preparation of these various derivatives, it must be conceded that this is only qualitatively true. With techniques now employed to effect acylation, nitration, alkylation, and the synthesis of other derivatives of starch, there is little doubt that some degradation of the starch molecule occurs.

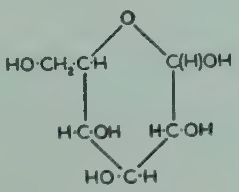
Because of its cheapness and commercial availability, starch has been employed in the past for uses that require a relatively small change in the starch molecule. More recently, however, starch has been examined for possible transformation into compounds in which a major change in its properties may be effected.

b. Structure of Starch. Any consideration of the derivatives of starch requires some discussion of the structure of the starch molecule. While this is dealt with in some detail in Chapter VIII, a very brief consideration of the topic here seems appropriate.

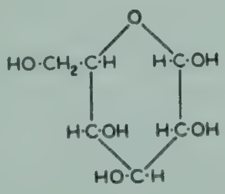
It is now known with a reasonable degree of certainty that (*a*) the starch molecule is built up of D(+)-glucose units (I), (*b*) these units exist in the pyranose ring (II), (*c*) the pyranose ring is in the α-form (III), and (*d*) the α-forms of these glucopyranose rings are bonded together through oxygen atoms in the 1,4 positions (IV) (49).



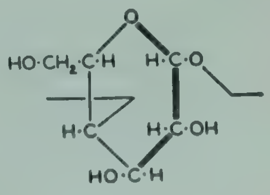
I. D(+)-Glucose



II. D(+)-Glucopyranose

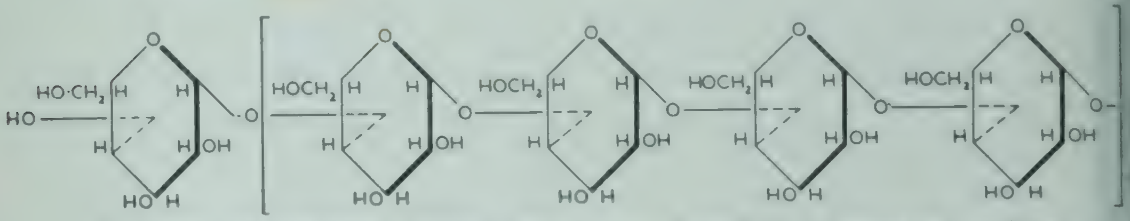


III α-D(+)-Glucopyranose



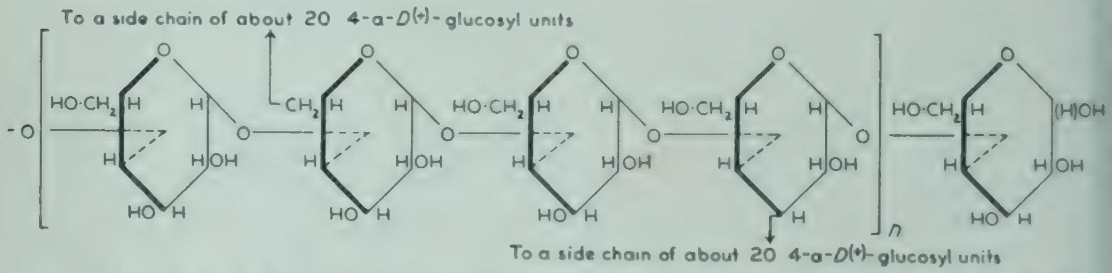
IV. α-D(+)-Glucopyranose residue

When the units indicated in (IV) are bonded together, a thread-like molecule of the type shown in (V) is obtained.



V. Thread-like molecule obtained from union of α-D(+)-glucopyranose residues through oxygen atoms in the 1,4 positions

Fractionation studies, however, have indicated that starch may be composed of two or more distinct varieties of molecules (168, 178, 221). One of these is generally conceded to be of the continuous chain variety shown in (V), whereas the other is thought to be of the branched chain variety shown in (VI).


To a side chain of about 20 4-a-D(+)-glucosyl units
-O
HO·CH₂
CH₂
(H)OH
n
To a side chain of about 20 4-a-D(+)-glucosyl units

VI. Branched chain molecule obtained from union of α-D(+)-glucopyranose residues through oxygen atoms in the 1,4 positions and periodic branching at positions 3 and 6

In view of available data on structure, a mixture of these two general types of molecules present the best current concept of the molecular structure of starch. Corn starch, for example, contains about 70% of the variety of molecule represented by formula VI and 30% of that represented by formula V, whereas corn starch from waxy maize is almost entirely of the type indicated by formula VI.

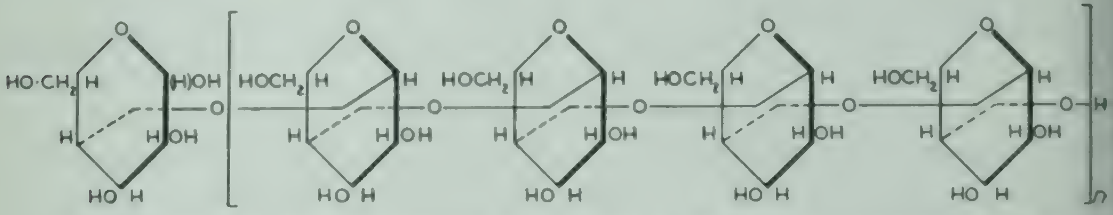
c. Pretreatment of Starch. The pretreatment of starch for the preparation of derivatives usually consists of (*a*) the removal of fatty material and phosphorus (148) and (*b*) the disintegration and or swelling of the starch granule (271). The fatty material and a portion of the phosphorus may be removed by extraction with 85% methanol (222) or ethanol which contains a small amount of nitric acid (148). The disintegration or swelling of the granule may be accomplished by use of alcohol (233), aqueous chloral hydrate (118), aqueous pyridine (81, 110, 168, 179), formic acid (256), hot glycerol (271), hydrogen chloride (192), hydrogen peroxide (238), phosphoric acid (237), or water (100, 185).

Alcohol is frequently used to precipitate starch from its aqueous solution. In some instances the freshly precipitated starch, still wet with alcohol, has been esterified, but this procedure is not recommended as the alcohol is esterified preferentially (233). The starch from the alcohol precipitation may be isolated as a dry fluffy powder by (a) pouring the starch paste into five or more times its volume of alcohol with vigorous stirring, (b) removing the remaining traces of water by filtering, stirring in fresh portions of alcohol and filtering, and (c) working at all times so as to prevent the adsorption of atmospheric moisture (259).

The preheating of starch granules in aqueous chloral hydrate or water results in swelling, disorganization, disintegration, and partial solution (118). Little or no degradation accompanies this treatment (160a). Special precautions must be used during the isolation, however, or a horny, non-reactive product is obtained. Acetone may be used to precipitate the starch from the aqueous solutions of chloral hydrate (160a).

Swelling or disorganization of the starch granule, with little or no degradation, is effected by the use of aqueous pyridine (81, 110, 168, 179). The greatest swelling occurs in 30% aqueous pyridine (108). A newer procedure uses 60% pyridine to swell starch, and then distills the water as a pyridine-water azeotrope (60 to 40% composition), to give an anhydrous gelatinized starch (168). Pretreatment with formic acid transforms starch into a more easily esterifiable product but doubtless causes some chemical degradation or alteration of the starch molecule (256). Similar effects are obtained in general by other acids, concentrated solutions of acid salts, oxidizing agents, or high temperatures. Comparable results are obtained by the use of hot glycerol, hydrogen chloride (192), hydrogen peroxide (238, 239), 85% phosphoric acid (237), or water (100, 185).

d. General Consideration of Derivatives. With the concept of the starch molecule pictured in formulas V and VI tentatively agreed upon, it becomes apparent that there are from two to four hydroxyl groups per glucose unit available for such reactions as acylation, nitration, and alkylation. Barring any appreciable amount of degradation during the process of derivative formation, starch might be expected to give two rather well defined series of acyl, nitro, and alkyl derivatives. The one series resulting from the configuration of V should resemble rather closely the corresponding derivatives of cellulose, inasmuch as Formula V is not dissimilar to VII, which represents the generally accepted structure for cellulose. The other series resulting from the configuration of formula VI should have a somewhat different set of properties. That experimental results indicate two such series will be shown in the subsequent discussion.



VII. Thread-like molecule obtained from union of β-D(+)-glucopyranose residues through oxygen atoms in the 1,4 positions to give the configuration of the cellulose molecule

2. Acyl Derivatives of Starch

a. Historical Review (32). The story of the esters of starch has its beginning in 1865 when Schützenberger obtained two acetyl derivatives (223). One of these he found to be soluble in both alcohol and acetic acid but insoluble in water. The saponification of both derivatives yielded a dextrin. In 1869, he heated starch with acetic anhydride at 140°C. and obtained an acetyl derivative which was hydrolyzed to regenerated starch (223). Similar results were obtained at 150°C., but at higher temperatures a dextrin was obtained as the hydrolytic product of the acetylated starch. Both the elementary composition and the rotatory power of these derivatives were determined. In 1870, he reported that acetylation of starch at 140°C. yields the triacetate of starch but that at 150°C. the triacetate of soluble starch is the principal product (223). This was confirmed by hydrolysis of the respective products to a starch-like substance and soluble starch.

The use of sulfuric acid as a catalyst in the acetylation of carbohydrates was first reported by Franchimont in 1879 (73). The oleic-sulfuric acid derivatives of both starch and dextrin were obtained by Liechti in 1883 (144). These products were found to be soluble in water, to yield metallic salts, and to be hydrolyzed with the regeneration of the carbohydrate and oleic acid.

Michael, in 1883, indicated that the starch granule is unaltered upon saponification after acetylation with acetic anhydride, while the presence of even small amounts of acetic acid in the acetylation mixture gives some degradation (163). The use of acetyl chloride was found to give an acylated dextrin.

In 1893, Cross treated soluble starch with alkali and then with benzoyl chloride and obtained the benzoate which he analyzed for carbon (44). Two years later Boettinger noted the production of an ester when starch was treated with an excess of glyoxylic acid (23).

Skraup suggested in 1898 that low temperature and low concentrations of sulfuric acid yield more complex products in the acetylation of starch than are obtained when high temperatures and high concentrations of sulfuric acid are used (227). Syniewski reported during the same year that he obtained an ester by acetylating starch with acetyl chloride and barium carbonate (239).

Pregl, in 1901, treated soluble potato starch with acetic anhydride (192). The derivative had a molecular weight about 10 times that of the empirical formula, had a specific rotation of 163.6°, and was insoluble in alcohol. Hydrolysis regenerated the starch. Acetylation of the same type of starch with an increased amount of sulfuric acid gave a triacetyl derivative. This product had a molecular weight about 3 times that of the empirical formula, melted at 150°C., was soluble in alcohol, and was hydrolyzed to yield a dextrin.

In 1904, Kldiashwili reported treating starch with formic acid to give the monoformyl ester (136). Cryoscopic measurements indicated a product which contained 6 glucose units. Comparable derivatives were obtained by treating starch with the mono-, di-, and trichloroacetic acids. In the following year he refluxed rice starch with twice its weight of dichloroacetic acid to give a derivative which, according to determinations of molecular weight, contained about 6 glucose units.

Cross *et al.*, in 1905, reported that they prepared the lower acetyl derivatives by heating starch in acetic acid at 100°C. (46). Their work indicated that the extent of acetylation is proportional to the time of heating and the ratio of acetic acid to starch. They described the lowest member of the series as exhibiting the characteristic physical properties of gelatin, and as drying to form a transparent and elastic membrane.

In 1905, Skraup treated starch with acetic anhydride saturated with hydrogen chloride and obtained a number of chloroacetylated starches (227). The chlorine content indicated a minimum molecular weight of 7440.

Law, in 1908, reported that neither acetylation nor hydrolysis occurs when starch is treated with acetic acid and acetic anhydride in the presence of a large excess of zinc chloride, whereas cellulose under the same conditions yields the triacetate (140). In the following year Traquair published a report on the preparation and properties of the formyl and acetyl derivatives of starch (248). He reported, furthermore, on the manufacture, properties, and uses of a product called "feculose," which he prepared by the acetylation of starch with glacial acetic acid or acetic anhydride at 90°C.

A review entitled *Stärkeazetat* was published by Worden in 1913 (265). In 1916 Boeseken, *et al.*, published on the velocity of acetylation of wheat starch (22). Of the various catalysts examined, they considered hydrogen iodide to be the best. They observed, furthermore, that the rate of acetylation is increased with increasing amounts of sulfuric acid as the catalyst, but that the increase in rate is not proportional to the increase in the amount of sulfuric acid used.

A second review entitled *Stärkeacetat* was published by Halen in 1921 (95). Karrer and Fioroni, in 1922, calculated the heat of esterification of starch to starch triacetate as 1306.0 k.-cal. (127). Experimentally they found a value of 1296.5 k.-cal. per mole.

In 1922, Pringsheim and Lassmann treated glycogen and the soluble starch of Zulkowski (degraded by heating in glycerol) with acetic anhydride in pyridine (195). From a consideration of the properties of the derivatives as well as those of the regenerated starting materials, they concluded that starch and glycogen are not identical.

In 1923, Escales and Levy reported that starch is not appreciably degraded when acetylated with sulfo fatty acids (Twitchell reagent) below 80°C. (57). They observed that the acetyl derivative of starch is very hygroscopic and that it is less viscous than the corresponding derivative of cellulose.

Gault, in 1923, reported the preparation of the lauryl ester by treatment of starch with the acid chloride in the presence of pyridine (84). He obtained a water-soluble derivative. Karrer (1923) reported on the treatment of glycerol-degraded starch (soluble starch of Zulkowski) with an acid chloride and quinoline for 4 hours (129). He prepared and characterized what he called the starch hexapalmitate and the starch hexastearate.

According to a patent issued in 1923, the higher esters such as the palmitate, stearate, and undecylate may be prepared by treatment of the unmodified starch with the appropriate acyl chloride in the presence of a tertiary base and a diluent under reflux (59). In the same year Berthon described the preparation of the higher esters by pretreatment of the starch with a mixture of benzene and pyridine, followed by heating with the appropriate acyl chloride in the presence of a tertiary base (20). In 1924, a procedure was patented for the production of the linoleic acid and similar esters of starch by treatment of the unmodified starch with the appropriate acyl chloride in the presence of a tertiary base. (60).

In 1924, Chowdhury treated starch with chloroacetic acid in the presence of sodium hydroxide and obtained a derivative which contained 2 hydroxy acid residues per glucose unit (37). This product was then methylated with methyl sulfate to give a mixed ether-ester. In the same year Gault prepared and described the diesters of starch (85).

A patent issued in 1926 covers the use of certain esters of starch in coating compositions (157). "Livering" and gelling are prevented by the use of a small amount of an acid such as acetic, citric, formic, malic, oxalic, phosphoric, or tartaric.

In 1927, Bergmann and Knehe degraded potato amylose by heating it in glycerol and then acetylated it by treatment with acetic anhydride in pyridine (13). According to patents issued during the year and subsequently, the higher esters of starch may be prepared by first making a starch paste, then converting to the alkaline starch, and finally treating with the appropriate acyl chloride (64). Peiser, in 1927, reported the treatment of dry starch with acetic anhydride in the cold to give an acetyl derivative, which was then treated with phosphorus pentachloride in toluene at 105°C. (185). The resulting product was a chlorinated acetyl derivative of starch.

Acetyl derivatives may be prepared by treating alkali-starch with acetic acid vapors, or by pretreatment of the starch with ammonia gas, formaldehyde, pyridine, or steam and subsequent treatment with acetic acid vapors, according to patents issued in 1927 (62).

In 1928 Friese *et al.* published the results of studies in which they degraded potato starch by heating in glycerol and then acetylated the degraded product with acetic anhydride in pyridine (81). In the same year they reported the preparation of the triacetates of both "amylose" and "amylopectin" of wheat starch. The derivative of the former was much more soluble in organic solvents than was that of the latter. Other workers have not confirmed these results (25, 242).

Haworth *et al.* (100) reported the acetylation of starch with chlorine and sulfur dioxide as the catalyst (Barnett catalyst (8)). The nature of the final product was not affected appreciably by the temperature of the reaction. Using zinc chloride as a catalyst, Tsuzuki (1928) obtained results which he believed indicated the absence of depolymerization during the acetylation process (250). With the zinc chloride catalyst, he also acetylated starch with acetic anhydride in non-aqueous solvents such as glycerol. He found, furthermore, that alkaline thiocyanates act catalytically in the acetylation of starch.

Hess and Smith and also Brigl and Schinle (193), in 1929, reported on the precipitation of rice starch paste with alcohol and the subsequent acetylation of the precipitated product (109). The resulting derivative was found to be soluble in organic solvents. Brigl and Schinle observed, moreover, that the product obtained by treating starch with acetic anhydride in pyridine in the absence of sulfuric acid is different from that obtained by similar treatment in the presence of acid (25). Karrer and von Krauss (1929) precipitated the starch paste with alcohol and acetylated the precipitate to obtain an ester which was soluble in chloroform (128). Patents for the use of sulfur dioxide as an acetylation catalyst were issued during 1929 and 1930 (62). Stein obtained a patent in 1931 for pretreating starch with a liquid swelling agent below 100°C. and then effecting acylation with an anhydride of the desired acid in the presence of a catalyst such as perchloric acid or thionyl chloride (235).

Clark and Gillespie, in 1932, reported the preparation of acetyl derivatives of starch by the use of glacial acetic acid at the reflux temperature of the solution with or without sodium acetate as a catalyst (38). They observed that a slower reaction rate resulted from the use of sodium acetate. Hughes *et al.* in 1932 reported securing acetyl derivatives readily by the pretreatment of starch with boiling water and subsequent drying with ethanol, followed by addition of acetic anhydride and a trace of sulfuric acid (118). For the production of acetyl derivatives of starch with low viscosities, Snelling and Boyd in a patent obtained in 1932 claim degradation by the use of a concentrated solution of ammonium nitrate with a boiling point greater than 110°C. (228).

Maltose octaacetate was obtained by Sutra (1932) when he treated starch with acetic anhydride at 70°C. (237). In 1933, Reich and Damansky reported the treatment of starch with cinnamoyl chloride in the presence of pyridine (199). Both the di- and triesters were obtained in good yields. Sutra suggested (1933) that both phosphoric and sulfuric acids cause degradation of the starch molecule (237).

Lorand obtained a patent in 1934 covering the use of sulfuryl chloride or magnesium perchlorate as a catalyst in the production of esters of starch (153). In the same year Damansky indicated that the use of sulfuric acid as a catalyst in acetylation reactions causes degradation of the starch molecule into "amylose" and "biose" (48).

In 1936, Genin reported pretreatment of starch by swelling and subsequent acylation by reaction with the anhydride of the desired acid (86). He prepared both stearic and various acetic-stearic esters of starch. The film-forming properties of the mixed esters improved with an increase in the amount of the acetate substituent.

Reich and Damansky, in 1937, claimed that native starch yields the diester and that further acylation results in degradation (200). This claim has not been confirmed by other workers. Staudinger and Husemann (232), in the same year, and also Higginbotham and Richardson, in 1938 (110), reported that, on acetylation of untreated starch, derivatives which are soluble in both chloroform and tetrachloroethane are obtained. The latter workers concluded, moreover, as the result of their studies, that very little degradation takes place through pretreatment with pyridine but that extensive modification occurs when the Barnett catalyst is used in acylation reactions of starch.

Meyer reported in 1942 that corn "amylose" is readily acetylated to give the triacetate when treated with acetic anhydride in pyridine (160). He found, moreover, that the triacetate from an "amylose" of medium molecular weight gave a derivative with a molecular weight of 78,000 when osmotically determined in tetrachloroethane. His calculations give a limiting value of 1.7 at infinite dilution for the viscosity of a triacetate of cellulose with a molecular weight of 78,000, and 1.05 for the corresponding value of a comparable starch ester.

The types of starch that have been acetylated include banana (98), canna (97), corn (118, 131, 160a), horse chestnut (233), rice (12, 14, 25, 111), waxy corn (101), and wheat (22, 185, 232, 233).

b. Preparation of Acyl Derivatives of Starch. 1. With Acetic Acid, Acetic Anhydride, and Sulfuric Acid. Acetyl derivatives of starch may be prepared by pretreating 25 g. of corn starch with 25 ml. of acetic anhydride for 30 minutes, after which a mixture of 100 ml. of acetic acid is added (30). The sulfuric acid, diluted with 45 ml. of glacial acetic acid, is then added. The reaction mixture is allowed to stand at room temperature until esterification is complete. The ester is then precipitated in cold water, and the precipitate filtered, washed with alcohol, and dried. Some results are presented in Fig. 71.

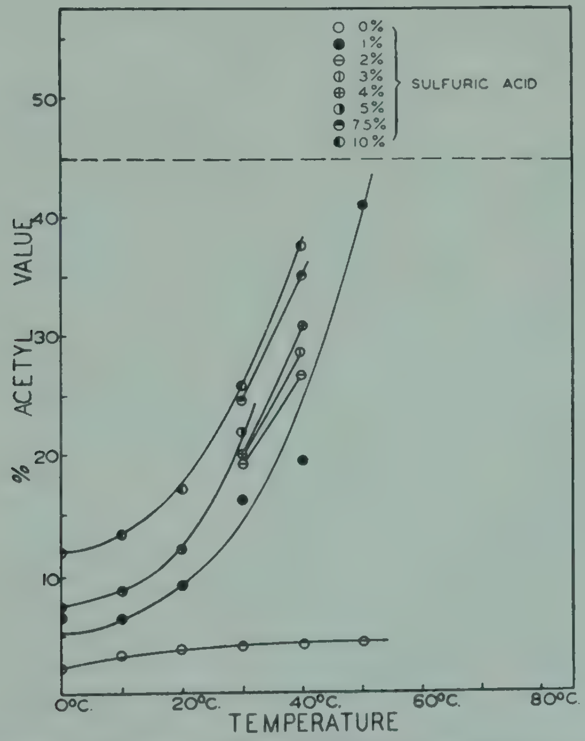


FIG. 71. Acetylation of starch. Effect of H_2SO_4 concentration and temperature on acetyl value.

The effect of time upon the acetyl value of the product is shown in Fig. 72 which indicates a short induction period (30). At an acetyl value of about 35 to 40% the rate of acetylation begins to decrease. From the graph it is seen that the presence of glacial acetic acid increases the rate of esterification over that reported by Boeseken.

The effect of various catalysts upon acetylation has been studied with regard to yield, reaction time, acetyl value (per cent acetyl, by weight), and product (30). The use of catalysts in the esterification of starch is known to cause degradation (25, 38, 80, 81, 95). In one procedure a small amount of catalyst is used at the reflux temperature of the acetylation mixture. Some results are summarized in Fig. 73. The products obtained after a 24 hour reaction period, with sulfuric acid and *p*-toluenesulfonic acid, had an acetyl value greater than that of maltose octaacetate. Sodium acetate apparently causes an increase in the rate of acetylation over that obtained without catalysts (*cf.* Fig. 72). This is contradictory to the observations of Clark and Gillespie (38).

The results shown in Figs. 71, 72, and 73 indicate that (*a*) the rate of acetylation is increased by the use of acetic acid in the acetylation mixture at both high and low temperatures, (*b*) the rate of acetylation at low temper-

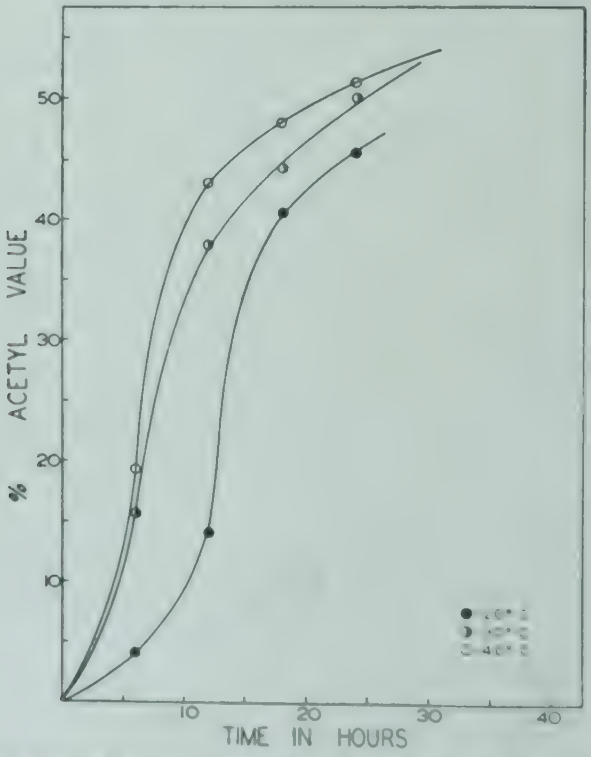


Fig. 72. Acetylation of starch. Change in acetyl value with reaction time at various temperatures.

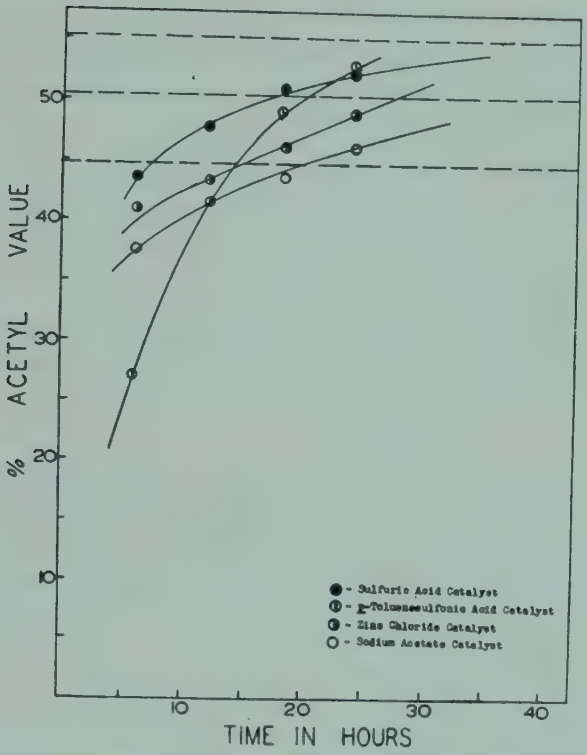


FIG. 73. Acetylation of starch. Change in acetyl value with reaction time when various catalysts are used.

atures is proportional to the temperature and the concentration of sulfuric acid, (*c*) the rate of acetylation increases rapidly after the initial induction period until an acetyl value of about 40% is attained, and (*d*) sulfuric acid is the most effective catalyst of the four studied.

The method used in measuring the acetyl value of the esters consists in titrating 1 g. of starch acetate in 50 ml. of carbon dioxide-free water with 0.1 *N* sodium hydroxide until alkaline to phenolphthalein (30). This is a measure of the free acid value. Twenty-five milliliters of 0.5 *N* sodium hydroxide is then added, the ester dissolved, and the solution boiled for 5 minutes. The excess base is neutralized with 0.5 *N* sulfuric acid and the acetyl value of the ester calculated.

The product known as feculose is obtained by the treatment of starch with glacial acetic acid at 90°C. (248), but acetylation may be effected even at a temperature of 80°C. (246).

2. *By Gelatinization with Pyridine and Acylation by Removal of Water as a Pyridine-Water Azeotrope.* A suitable procedure is to boil the native starch in water and then add pyridine and continue the boiling with distillation so as to eliminate the water as pyridine-water azeotrope boiling at

92-93°, thereby producing a solution or paste of starch in pyridine (179). The products so obtained range from clear solutions, when the pyridine contains a small amount of water (about 4%), to thick jellies when water is absent.

The starch gelatinized in tertiary bases is highly reactive toward esterification reagents such as the anhydrides and acyl halides of aliphatic and aromatic acids and is found to be trifunctional, giving rise to triesters in practically quantitative yield.

After gelatinization, the starch is treated with acetic anhydride to give acetic acid and effect acetylation. The esters are then isolated by the precipitation procedure. Acetates, propionates, and butyrates have been prepared by this procedure.

To determine acyl content, 0.5 to 1.0 g. samples are placed in a glass-stoppered flask, covered with 10 ml. of methanol, and allowed to stand for an hour (169). An equal volume of water is then added and the mixture made alkaline with 20 ml. of 1 *N* sodium hydroxide. This solution is allowed to stand for 12 hours, with occasional agitation, and titrated back with 0.5 *N* hydrochloric acid.

3. *By Use of an Acid-Anhydride Mixture at Reflux Temperature.* Starch acetates are prepared also at elevated temperatures by refluxing 25 g. of dried starch with a mixture of acetic acid and acetic anhydride (30). Forty-five milliliters of acetic anhydride are slightly more than sufficient to react with the moisture in the starch and the water of esterification. An excess of acetic acid is used so that it may function both as a reagent of the reaction and as a solvent for the ester. The product is precipitated in water and leached for 24 hours, after which it is filtered, washed, and dried. Some results appear in Fig. 74. The rate of acetylation is slow until an acetyl value of about 4% is attained, then very rapid, and finally slow but uniform until acylation is complete. The break in the esterification curve is accompanied by a clearing of the viscous solution. The results of changing concentrations and the lower reaction temperatures in these experiments show that a large excess of acetic anhydride is unnecessary. The lower reaction temperatures give starch acetates of lower acetyl values.

Starch dried at 110°C. has been treated with propionic acid and propionic anhydride under varying conditions with respect to temperature, time, and stirring (154). The results are summarized in Figs. 75 to 79.

4. *By Treating Finely Divided Starch with Acylating Agents under Mild Experimental Conditions* (259). The disintegration of the starch granules is effected by treating swollen starch granules suspended in water to the high shearing action of a Waring blendor for ½ hour, at a temperature of about

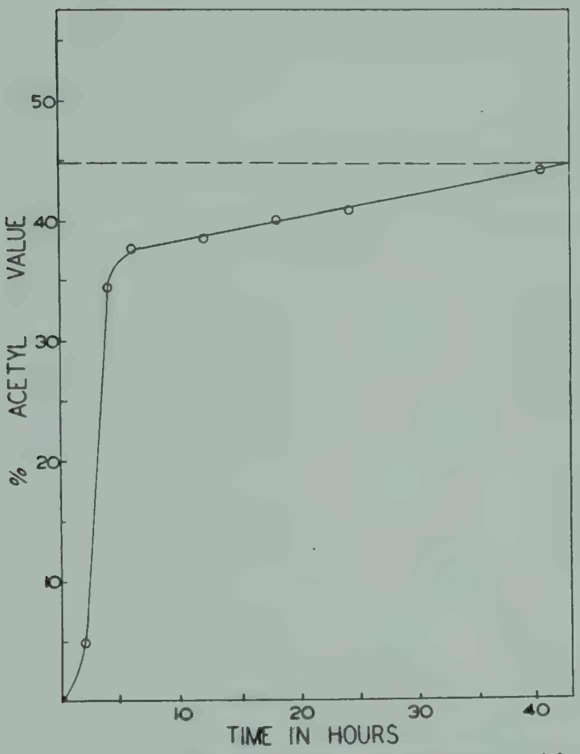


FIG. 74. Acetylation of starch at reflux temperature with no catalyst.

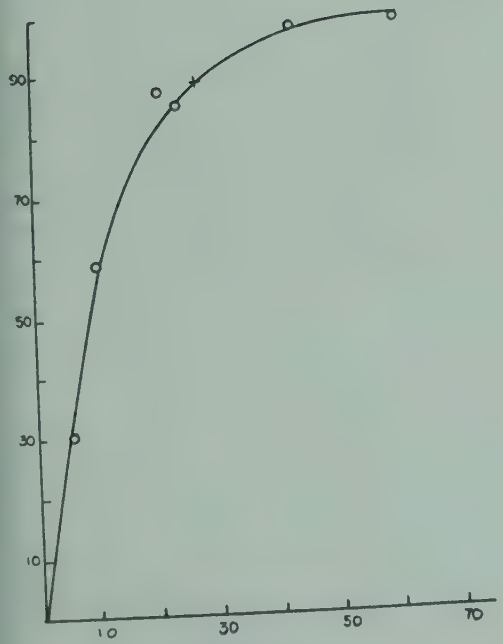


FIG. 75

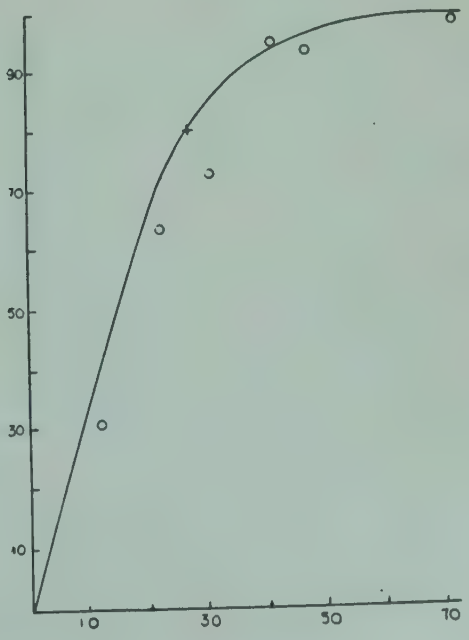


FIG. 76

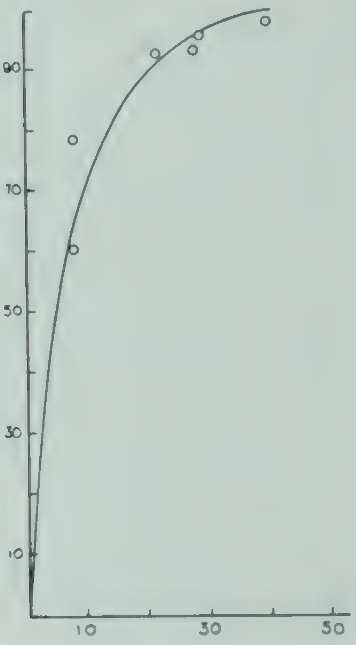


FIG. 77

FIGS. 75 to 77. Variation of esterification with time. The abscissa represents time in hours; the ordinate, per cent esterification. FIG. 75, Series I; FIG. 76, Series II; FIG. 77, Series III.

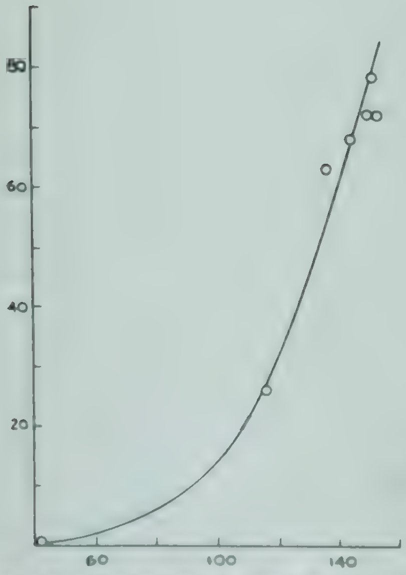


FIG. 78. Variation in esterification with temperature. Abscissa, temperature, °C; ordinate, per cent esterification.

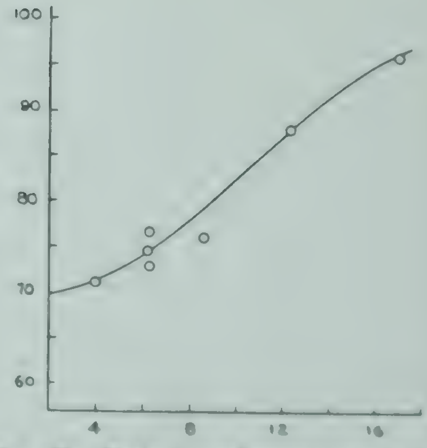


FIG. 79. Variation of esterification with stirring. Abscissa, speed of stirrer r.p.m./100; ordinate, per cent esterification.

75°C. This highly dispersed starch is then wholly precipitated by slowly pouring into a large volume of ethanol. The precipitate is then washed with ethanol and dried under reduced pressure to a fluffy powder.

The procedure recommended is to suspend 35 g. of fat-free starch in 500 ml. of distilled water and heat with constant stirring in a boiling water bath for 30 min. (259). The hot paste is then disintegrated in the bowl of a Waring blendor for 30 minutes at 12,000 r.p.m. at a temperature of 75°C. The mixture is cooled to 35°C. and poured into 5 parts of absolute ethanol in a Waring blendor. The precipitate is filtered and again dispersed in 300 ml. of absolute ethanol in a Waring blendor for three washings. The resulting finely divided precipitate is dried in a vacuum desiccator over anhydrous calcium chloride, and then in a vacuum oven at 70°C. to a moisture content of about 3 %.

The starch is weighed (about 50 g.), placed in a round bottomed 3-necked flask, fitted with ground joints, and 2.7 equivalents (4 parts by weight) of dry pyridine and 1.7 equivalents (3.25 parts by weight) of acetic anhydride are weighed in successively (259). A mercury-sealed stirrer is fitted to the center neck of the flask and a reflux condenser protected by a calcium chloride tube is placed in one of the side necks. The third neck is sealed by a glass stopper. The stirrer is started and the flask heated in an oil bath at 100°C. Within 10 to 15 minutes the starch dissolves and produces a highly viscous solution. After 3 to 6 hours the flask is removed, cooled to about 20°C., and the contents poured into 2 liters of ethanol while being stirred in a Waring blendor. The starch acetate which is precipitated in the form of white, curdy flakes is filtered on a hardened filter paper or fritted glass funnel, and again vigorously stirred with a fresh 300 ml. portion of ethanol for three successive washings. The acetate is allowed to stand in 300 ml. of ethanol overnight, filtered, and dried in a vacuum desiccator over calcium chloride. The resulting product is a fine white powder.

The effect of temperature and time on the progress of acetylation of very finely divided starch is shown in Figs. 80 and 81.

5. *By Use of Ketene on Untreated Starch.* Glucose and some of its derivatives have been acetylated with ketene (119). Ketene, in the presence of an acid catalyst such as sulfuric acid (30, 164) or hydrogen chloride (30), produces a low degree of acetylation of raw starch. Somewhat comparable results are obtained with ketene in the presence of traces of chlorine (30). The acetylation of starch with ketene was studied to determine the type of modification produced by small amounts of ketene (30). Two general methods of acetylation were used: a liquid phase reaction in ether and in acetone, and a solid-gas phase reaction which was carried out in a Pyrex tube (2.5 ft.

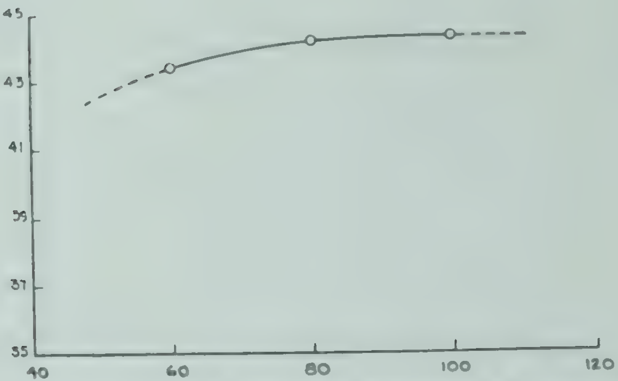


FIG. 80. Effect of temperature on extent of acetylation of corn starch; acetylation period, 6 hours. Abscissa, temperature, °C.; ordinate, per cent acetyl.

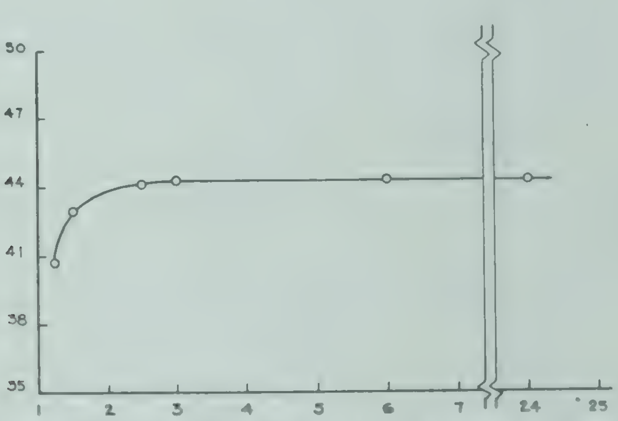


FIG. 81. Effect of time on extent of acetylation of corn starch; temperature, 100°C. Abscissa, time in hours; ordinate, per cent acetyl.

by 14 mm.). Hydrogen chloride and chlorine were used in several acetylations to increase the rate of reaction of ketene upon the starch in the solid-gas phase acetylations. Table XVI contains the results of these experiments. Tests show that these products have filming properties different from those of starch.

6. *Catalysts.* The use of catalysts for esterification of raw starch is known to effect an appreciable amount of degradation (25, 38, 80, 81, 95). When the Barnett mixture is used (8), for example, the product has the reducing power, solubility, and viscosity characteristics of a partially degraded starch (97, 100, 110). The use of ammonium nitrate as a catalyst, at or above 110°C., gives degraded starch acetates of low viscosities (228). Sulfuric acid was used as an esterification catalyst for the carbohydrates as early as 1879 (73). The rate of acetylation is increased by an increase in the concentration of the sulfuric acid, up to a certain limit and by an in-

TABLE XVI

Starch Acetate Prepared with Ketene

Experiment No.	Solvent	Volume of solvent	Reagent added	Amount added	Temperature	Time	Color	Free acid	Acetyl value
		ml		drops	°C.	hrs.		per cent	per cent
1	Ether	75	H_2SO_4	3	25	0.5	White	0.36	9.43
2	Ether	90	H_2SO_4	4	25	1	White	0.18	3.72
3	Acetone	70	H_2SO_4	4	25	2	White	0.12	2.18
4	Acetone	80	H_2SO_4	4	56	2	White	0.23	3.52
5	None		None		25	0.5	White	0.12	3.08
6	None		None		25	1.5	Tan	0.39	9.05
7	None		Cl_2	Saturated	25	2	Tan	0.32	8.05
8	None		HCl	Saturated	25	2	Tan	0.07	7.43

crease in temperature (30, 118, 227). Acid catalysts other than sulfuric acid are usually less effective and their use is accompanied by considerable degradation, but both sulfuric acid and phosphoric acid cause degradation of the starch molecule (48, 237). Potassium and sodium thiocyanates, as catalysts, are about as effective as is sodium acetate (250-252). *p*-Toluenesulfonic acid is an effective catalyst but has a very long induction period (30). Glacial acetic acid appears to increase the rate of esterification (30). The effect of time varies with the type of catalyst (30). Other catalysts which have been used include magnesium perchlorate (153), sulfuryl chloride (153), and the Twitchell reagent (153).

7. *Pretreatment.* Starch has been pretreated for esterification by the use of acetic acid (30), acetic anhydride (30), alkali (62), glycerol (13, 25, 81, 195, 242, 250), heat (192, 194), mechanical disintegration (25, 81, 110, 179, 199, 200), pyridine (25, 81, 110, 179, 199, 200), and water (118, 185, 197). Acetylating reagents which have been used on *pretreated* starch include acetic acid vapors (62), acetic acid with a trace of sulfuric acid (30), acetic anhydride with an alkali thiocyanate (250), acetic anhydride with perchloric acid or thionyl chloride (235), acetic anhydride with pyridine (13, 25, 81, 195, 242), acetic anhydride with sulfuric acid (100), acetic anhydride with zinc chloride (250, 251), acetyl chloride with pyridine (179), and acetyl chloride with quinoline (129).

8. *Other Acyl and Aroyl Derivatives of Starch.* The treatment of starch with formic acid at room temperature for 24 hours effects gelatinization and the formation of a monoformate ester, which is stained red-brown by iodine (89, 135, 136, 248). Comparable results are obtained by heating the mix-

ture, but the triformate is not obtained without continued treatment and consequent depolymerization (89, 135, 136, 248).

The mono-, di-, and trichloroacetyl esters of starch are prepared by heating starch either with the appropriate acid or with the acid anhydride in the presence of sulfuric acid (135, 136). The trichloroacetyl derivatives of starch may be prepared also by treating the triacetyl derivative of starch with phosphorus pentachloride at 135-140°C.

The glyoxylic ester of starch was prepared in 1895 by the treatment of starch with glyoxylic acid (23). It is obtained also when starch is treated with chloroacetic acid in the presence of aqueous sodium hydroxide (37).

A mixture of the propionyl derivatives of starch is prepared by refluxing starch with propionic acid and propionic anhydride (154, 169). The lower derivatives are water soluble whereas the higher acylated starches are soluble in organic solvents. The triester may be obtained also by treating pyridine-treated starch with propionic anhydride and distilling the water as a pyridine-water azeotrope (154, 169). A mixture of the butyryl derivatives of starch, mostly the triester, is obtained by treating pyridine-treated starch with butyric anhydride (169). The triester is soluble in organic solvents (169). The triproprionyl and the tributyryl derivatives of starch form brittle, clear plastics, which are somewhat softer than are those of the triacetyl derivatives (170). Similar results have been noted for the cellulose esters, which confirms the belief that the attraction between the starch molecules is rendered less effective by larger aliphatic substituents (170).

A mixture of the stearoyl esters of starch may be prepared by treating gelatinized starch with stearic anhydride (86, 152). Starch esters of lauric, myristic, and palmitic acids may be prepared similarly. Typical procedures are:

The dilauroyl ester of starch is prepared by heating one part of dry starch for two hours at 100°C. with five parts of lauroyl chloride in 2.5 parts of pyridine and 3 parts of toluene (84). The derivative is insoluble in acetone, alcohol, and water, but is soluble in benzene, chloroform and tetrachloroethane. A benzene-chloroform solution of this ester, on evaporation, forms a brittle film (84).

The tripalmitoyl ester of starch is prepared by heating 1 part of starch with 6 parts of palmitoyl chloride in quinoline at reflux temperature for about 3 hours (20, 152). The quinoline may be replaced by 4 parts of benzene and 1.8 parts of pyridine, the mixture refluxed for thirty minutes, and the partially esterified product precipitated with ethanol (20, 152).

The esters and mixed esters may be prepared by the use of the appropriate anhydride or mixture of anhydrides on pretreated starch (86, 109, 128). The film properties are improved with the use of increasing amounts of acetic anhydride (86, 109, 128).

The higher aliphatic esters of starch are usually prepared by treating starch with either the appropriate acyl halide or anhydride in the presence of alkali or a tertiary organic base (20, 59, 64, 94). Organic bases which have been used include dimethylaniline (205), picoline (152), pyridine (20, 59, 85) and quinoline (129).

A starch hexapalmitate and a starch hexastearate have been prepared and characterized (129), as have also oleic-sulfuric acid derivatives of both starch and dextrin (144).

A benzoyl-lauroyl ester of starch may be prepared by mixing 1 part of starch with 1 part of 40% sodium hydroxide solution, refluxing the mixture in benzene, and adding dropwise a mixture of benzoyl and lauroyl chlorides. Heating should be continued a few minutes after the last of the acyl chlorides is added.

A mixture of benzoyl esters of starch is prepared by heating starch in pyridine with benzoyl chloride for 72 hours at 75°C. (47, 199, 200). The triester is insoluble in alcohol and ether but is soluble in acetone, chloroform, and pyridine (47, 199, 200). The tribenzoyl ester may be produced also by allowing pyridine-treated starch to stand in benzoyl chloride at room temperature for about 12 days (107). The optical rotation of the triester in chloroform is $(\alpha)_D^{20} = 80°$ (107). This ester was prepared in 1893 by treating soluble starch with alkali and then with benzoyl chloride (44).

The di- and tricinnamoyl derivative of starch is prepared by heating dry starch in pyridine with cinnamoyl chloride at 75°C. (199, 200).

The tritosyl starch is prepared by treating a pyridine-treated starch with tosyl chloride (*p*-toluenesulfonyl chloride) in the presence of pyridine at room temperature for about nine days (107). This derivative decomposes at 235°C., is insoluble in most solvents, and yields 2, 3-ditosyl-6-iodostarch when treated with sodium iodide in acetone for 3 days at 100°C. (107). These derivatives, when treated with hydrogen bromide in glacial acetic acid, yield respectively 1-α-bromo-2,3,6-tritosyl-4-acetylglucose and 1-α-bromo-2,3-ditosyl-4-acetyl-6-iodoglucose (106).

The 3,4-dichlorobenzenesulfonyl ester and similar derivatives of starch may be prepared by treating dry starch with the appropriate aroyl chloride in the presence of an alkali or tertiary amine base (117).

c. Properties of Acyl Derivatives of Starch. (*31*). The acetyl derivatives of starch can be classified into three general groups: (*a*) water-soluble, insoluble in organic solvents; (*b*) water-soluble, soluble in organic solvents; (*c*) water-insoluble, soluble in organic solvents. The acetyl derivatives of starch are usually characterized by determination of their (*a*) acetyl value, (*b*) behavior in solution, (*c*) fusion temperature or melting point, (*d*) mechanical properties, (*e*) optical rotation, (*f*) solubility in various types of solvents, and (*g*) solubility and compatibility (190). The procedures normally used in estimating the molecular weight of acetylated starches include: cryoscopic (231, 250), diffusion (211, 212), ebullioscopic (231, 250), osmotic pressure (231), and viscosity measurements (72).

Although high polymers frequently give abnormal results with respect to molecular weight determinations by the cryoscopic method (75), this method has been used to some extent in the determination of the molecular weight of acetylated starches (231, 250). Even in the study of the derivatives of cellulose, the cryoscopic method may lead to erroneous results because of both the tendency of the solute to crystallize and the probable presence of impurities of low molecular weight (78).

The diffusion measurements on acetyl derivatives of starch in solvents such as acetic acid and acetic anhydride indicate a molecular weight of about 225,000 (211, 212). Ebullioscopic measurements for the estimation of the molecular weight of the esters of starch have proven unsuccessful (231, 250). The osmotic pressure measurements on solutions of acetylated potato starches suggest a molecular weight of from 45,000 to as much as 275,000 for the various fractions (231). Comparable measurements on the triacetyl derivatives of "amylose" and "amylopectin" in tetrachloroethane give values respectively of 78,000 and 300,000 (160 a).

The comparative flow times of the acetic acid solutions of acetyl derivatives of starch have been determined (57). The empirical formula (229, 232, 233), $\eta_{sp}/C = Km\,M$, relates the viscosity of a polymer in solution to its molecular weight. The specific viscosity, η_{sp}, is equal to the relative viscosity minus 1. The C is the concentration of the solution in basal moles per liter, Km is a constant for a given homologous series in a specific solvent, and M is the molecular weight (229). This formula is only relatively accurate (162). The viscosity of a number of dilute solutions of acetyl derivatives of starch have been measured (230, 234), and is relatively independent of temperature (231). The viscosity of a carefully prepared triacetyl ester of starch, in tetrachloroethane, however, is reported to vary appreciably with the temperature (110). The viscosity of the triacetyl ester of "amylose" is considerably higher than is that of the comparable ester of "amylopectin" (160a). When the Kraemer intrinsic viscosity (138) is plotted against concentration, for the triacetyl ester of potato starch, a straight line with a positive slope is obtained whereas the acetyl ester of cellulose gives a line with a negative slope (169). The significance of viscosity measurements on starch pastes has been considered (26).

The viscosity of aqueous solutions of acetylated starches has been measured by use of an Ostwald viscosimeter, at various concentrations, degrees of aging, and temperatures (31).

The melting point and/or decomposition temperature, specific gravity, optical rotation, viscosity, and solubility are included for six types of standard samples of acetates of starch (*cf.* Tables XVIII, XIX, and XX.).

The solubility of an esterified starch is dependent upon (*a*) the acyl value, (*b*) the degree of degradation of the esterified molecule, (*c*) the degree of polymerization of the ester, (*d*) the fractionation of the starch or the derivative, (*e*) the nature of the acyl substituent (*f*) the type of starch, and (*g*) the solvent and temperature.

Acetylated starches of low acetyl value (up to 25%) are reported to be water soluble (38, 46). If the derivatives are not degraded, however, it is likely that acetylated starches with acetyl values of 15% or above are not water soluble (190). The triacetyl derivatives of potato starch swell but are insoluble in acetone, benzene, chloroform, and ethyl acetate (81, 110).

The degree of solvation of an esterified starch is exceptionally high in a polar solvent such as formic acid (231). The "amylose" molecules are less extended than are the cellulose molecules and are believed to be arranged in a spiral (79), which accounts in part for their greater solubility. Acetylated "amylose" is quite soluble in ethyl acetate (160a).

The triacetyl derivatives of starch are soluble, in general, with the exception perhaps of potato starch, in acetic acid, chloroethane, chloroform, tetrachloroethane, and other halogenated hydrocarbons. They are insoluble in alcohol, ethyl acetate, and, probably, in acetone and pyridine. The triacetyl ester of waxy corn starch, perhaps on account of its low molecular weight, is soluble in a variety of solvents (190).

The melting point and/or decomposition temperature of acetyl derivatives of starch have been reported by several authors (239, 250, 265). In determining melting points, three temperatures were recorded (185): that at which a pseudoliquid or the first apparent melting occurs, that at which a semitransparent stage begins to appear, and that at which the entire mass becomes transparent. Further heating decomposes the samples. In the case of the water-soluble esters only a decomposition temperature is observed when the samples turn from white to brown.

The specific gravity was determined by means of a 25 ml. pycnometer. Methylcyclohexane was used as the inert liquid (245).

Data have been published concerning the specific rotation of acetyl derivatives of starch in various solvents (185, 192, 223, 250, 265). Table XVIII contains some specific rotations. These measurements on the esters of starch, as well as those on raw starch, indicate that the β-type of linkage does not occur to any appreciable extent in the starch molecule (161).

TABLE XVII

Melting Point, Specific Gravity, and Optical Rotation (31)

Sample No.	Type of ester	Free acid	Acetyl value	Ash	Melting point: First melting or decomposition	Melting point: Transparent	Specific gravity, 25°C.	$[\alpha]^{20}_{D}$
		per cent	per cent	per cent	°C.	°C.		degrees
11	5-7%, low viscosity	0.30	6.60	0.26	240-246		1.64	+225.0*
12	5-7%, high viscosity	0.36	4.90	0.03	235-268		1.59	+220.1*
13	9-11%, low viscosity	0.30	9.30	0.20	238-258		1.55	+188.6*
14	9-11%, high viscosity	0.30	9.80	0.04	250-260		1.57	+180.0*
15	Triacetate, low viscosity	0.20	42.90	0.14	188	205-212	1.24	+166.7†
16	Triacetate, high viscosity	0.02	42.20	0.02	175	220	1.20	
25	Triacetate, low viscosity	0.02	41.00		181	200-202		
26	Triacetate, high viscosity	0.02	41.60		180	195-215	1.15	+172.5†

*Water was used as solvent.
†Chloroform was used as solvent.

TABLE XVIII

Optical Rotation of Acetylated Starches

Acetyl ester of	$[\alpha]_D$	0°C.	Solvent used	Investigators
Banana	+167°	20°	Chloroform	Hawkins, Jones, and Young (98)
Canna	164	—	Chloroform	Hassid and Dore (97)
Corn	156.5	22	Pyridine	Mullen and Pacsu (169)
Corn, 5-7%, L.V.*	225	20	Water	Burkhard and Degering (31) No. 11
Corn, 5-7%, H.V.†	220.1	20	Water	Burkhard and Degering (31) No. 12
Corn, 9-11%, L.V.	188.6	20	Water	Burkhard and Degering (31) No. 13
Corn, 9-11%, H.V.	180.0	20	Water	Burkhard and Degering (31) No. 14
Corn, Tri-, L.V.	166.7	20	Chloroform	Burkhard and Degering (31) No. 15
Corn, Tri-, H.V.	172.5	20	Chloroform	Burkhard and Degering (31) No. 26
Potato	177-9	18	Chloroform	Baird, Haworth, and Hirst (100)
Potato	168.4	20	Chloroform	Brigle and Schinle (25)
Potato	158	22	Pyridine	Mullen and Pacsu (169)
Potato	176.3	23	Chloroform	Reich and Damansky (199, 200)
Potato	170	19	Chloroform	Hirst, Plant, and Wilkinson (100)
Potato, amylose	170	19	Chloroform	Hirst, Plant, and Wilkinson (100)
Potato, amylose	172	20	Chloroform	Freudenberg and Rapp (78)
Rice	162.4	19	Chloroform	Brigle and Schinle (25)
Rice	171	20	Chloroform	Hirst and Young (111)
Rice	157.9	22	Pyridine	Mullen and Pacsu (169)
Tapioca	155	22	Pyridine	Mullen and Pacsu (169)
Waxy corn	166	18	Chloroform	Haworth, Hirst, and Woolgar (104)
Wheat	151	22	Pyridine	Mullen and Pacsu (169)

*L.V. = Low viscosity
†H.V. = High viscosity

TABLE XIX

Effect of Temperature of Preparation of Solutions of Starch Acetate upon Viscosity

Temperature for preparation of solution	Concentration of solution	Density at 25°C.	Time	Viscosity
°C.	per cent		sec.	millipoise
25	5.12	1.018	113.5	22.58
	5.88	1.021	130.0	26.01
	9.23	1.035	238.5	48.7
	11.73	1.046	360.0	74.5
50	4.64	1.015	112.2	22.23
	9.30	1.035	270.0	55.22
	13.94	1.055	671.0	140.2
	13.17	1.052	500.0	104.2
100	4.65	1.0148	108.8	21.53
	9.54	1.0336	265.0	54.12
	14.07	1.0520	585.0	121.9
	18.72	1.0723	1329	282
	24.69	1.0936	3234	700

Escales and Levy have studied the comparative flow times of acetic acid solutions of acetyl derivatives of starch (57). Staudinger *et al.* have reported the viscosities of some dilute solutions (230-232, 234). Some viscosities of solutions in water, or in acetone determined in an Ostwald viscosimeter, are reported in Tables XIX and XX.

To determine the effect of age upon the viscosity of solutions of the acetyl derivatves of starch, the pH of the solutions was determined at the time of measuring the viscosity. To prevent molding, a crystal of mercuric iodide may be added to the water solutions of the derivatives of starch.*

The water-soluble esters gave a floc when attempts to dissolve them were made. This was overcome by heating the water to 100°C. before the ester was added and then cooling rapidly as soon as solution was effected. The viscosity of the solutions prepared at 100°C. was the same as those prepared at lower temperatures (*cf.* Fig. 82). All the water-soluble esters were put into solution at 100°C., and no floc appeared even after 5 months (*cf.* Table XIX).

Viscosities are calculated from the equation

$$\eta = Adt - \left(\frac{Bd}{t}\right)$$

---

*Mercuric iodide was found to stabilize the solutions against mold very effectively. Other agents which were tested, but later rejected because of certain physical properties that made them poor agents to add, include formaldehyde, toluene, benzene, acetaldehyde, lead acetate, and xylene.

TABLE XX

Viscosities and Densities of Starch Acetate Solutions

Starch Acetate No.	Age of solution	Concentration of solution	Density at 25° C.	Time	Viscosity at 25° C.	pH reading
		per cent		*sec.*	*millipoise*	
11. 5-7%, low viscosity	Fresh	5.11	1.0173	202.0	40.53	3.95
		9.77	1.0369	714.0	146.6	3.60
		13.45	1.0542	1961.0	410	3.45
		18.80	1.0768	7380.0	1574	3.9
		23.17	1.0870			3.6
	1 wk.	5.11	1.0176	187.0	37.50	3.9
		9.77	1.0360	628.0	128.9	3.8
		13.45	1.0545	1627.0	340	3.7
		18.80	1.0768	6107.0	1299	3.8
	5 mos.	5.13	1.0175	166.2	33.27	3.35
		9.90	1.0288	546.0	111.3	3.25
		14.14	1.0548	1435.8	300	3.20
		19.21	1.0775	6327.0	1350	3.7
		24.01	1.0966			3.2
12. 5-7%, high viscosity	Fresh	5.16	1.0175	896.0	180.6	3.60
		9.18	1.0363	5678.0	1165	3.55
		14.10	1.0569			3.70
	1 wk.	5.16	1.0170	573.0	115	3.8
		9.18	1.0363	2857.0	586	3.5
		14.10	1.0569			3.6
	5 mos.	5.10	1.0180	332.2	66.88	3.15
		9.79	1.0368	1676.4	344	2.90
		13.78	1.0539	7455.0	1556	3.20
13. 9-11%, low viscosity	Fresh	5.56	1.0179	140.2	28.00	3.55
		9.72	1.0357	335.6	68.75	3.5
		13.75	1.0563	828.0	173.2	3.7
		18.28	1.0735	1727.0	367	3.6
		23.33	1.0982	4718.0	1026	3.6
	1 wk.	5.56	1.0189	137.6	27.55	3.8
		9.72	1.0353	320.0	65.55	3.6
		13.75	1.0561	769.0	160.8	3.3
		18.28	1.0735	1587.0	337	3.6
		23.33	1.0981	4396.0	1022	3.5
	5 mos.	5.39	1.0183	133.7	26.68	2.90
		9.77	1.0363	310.6	63.66	2.65
		14.54	1.0710	774.4	162.2	2.80
		18.35	1.0749	1664.0	345	3.0
14. 9-11%. high viscosity	Fresh	5.33	1.0173	142.6	28.50	3.80
		9.74	1.0359	364.0	74.60	3.45
		13.98	1.0542	879	183.5	3.85
		17.68	1.0701	1797.0	381	3.7
		22.99	1.0961	5160	1120	3.6
	1 wk.	5.33	1.0169	139.8	27.95	3.8
		9.74	1.0336	350.0	71.75	3.6
		13.98	1.0541	830	173	3.3
		17.68	1.0700	1710.0	362	3.6
		22.99	1.0965	4794.0	1041	3.5

TABLE XX—*Concluded*

Starch Acetate No.	Age of solution	Concentration of solution	Density at 25°C.	Time	Viscosity at 25°C.	pH reading
		per cent		*sec.*	*millipoise*	
14. 9-11%, high viscosity	5 mos.	5.25	1.0179	136.8	27.31	2.95
		9.85	1.0365	353.0	70.33	2.75
		14.29	1.0545	819.0	180.0	2.80
		18.50	1.0737	1782.0	379	3.1
		24.37	1.0975	5290.0	1150	3.1
Starch Triacetate No.						
15. Low viscosity	Fresh	5.22	0.8042	75.4	11.62	6.1
		10.16	0.8230	237	38.51	5.7
		15.17	0.8423	666	111.1	5.5
		20.41	0.8665	2016	346	5.4
		24.73	0.8872	4841	851	5.4
	1 wk.	5.06	0.8045	75.4	11.62	6.1
		9.85	0.8248	236.6	38.50	5.6
		14.49	0.8434	654	109.2	5.4
		21.03	0.8692	1998	344	5.3
		23.33	0.8840	4661	816	5.3
	5 mos.	5.50	0.8058	74.0	11.42	5.9
		10.37	0.8241	232	37.75	5.4
		20.34	0.8658	1648	282	5.1
		25.76	0.8887	2870	505	5.0
		27.51	0.8963	9900	1770	4.9
16. High viscosity	Fresh	5.28	0.8044	140.2	22.13	6.1
		10.13	0.8220	636	103.7	5.7
		16.15	0.8434	3820	638	5.5
		19.70	0.8666	11320	1940	5.4
	1 wk.	5.25	0.8032	136.8	21.57	6.1
		9.74	0.8228	645	105.2	3.6
		14.16	0.8450	3146	526	5.4
		18.62	0.8646	7781	1333	5.3
	5 mos.	5.58	0.8065	117.8	18.57	5.9
		10.52	0.8250	439	71.8	5.4
		16.20	0.8459	2485	439	5.1
		22.06	0.8732	9910	1715	5.0
26. High viscosity	Fresh	4.78	0.8027	99.2	15.48	6.1
		9.69	0.8231	408	66.6	5.7
		14.18	0.8418	1495	249	5.5
		18.38	0.8633	4748	812	5.4
		26.87	0.8893	14155	2495	5.4
	1 wk.	4.82	0.8036	99.0	15.47	6.1
		9.83	0.8238	421	68.6	5.6
		14.51	0.8438	1476	246	5.4
		18.60	0.8650	4655	797	5.3
		24.27	0.8882	12645	2225	5.3
	5 mos.	5.45	0.8061	105.2	16.52	5.9
		18.56	0.8571	2118	360	5.4
		19.32	0.8585	2700	459	5.1
		22.47	0.8742	5745	995	5.1
		32.81	0.9216			4.9

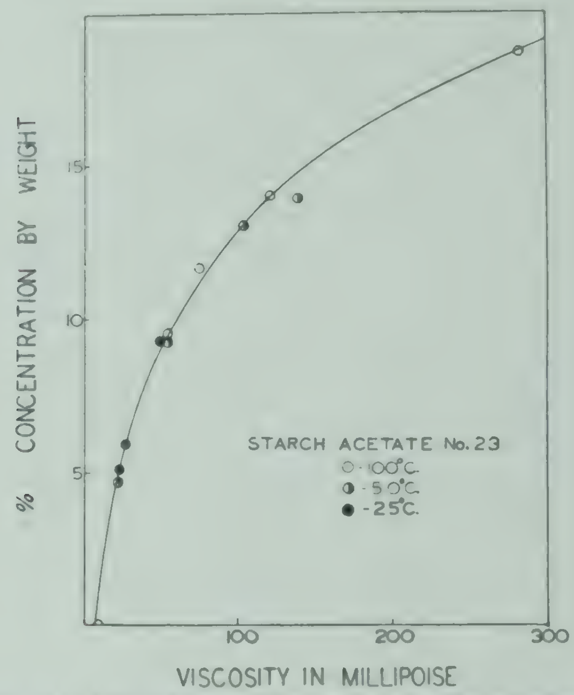


FIG. 82. Effect of solution temperature upon vicosity of starch acetate solutions

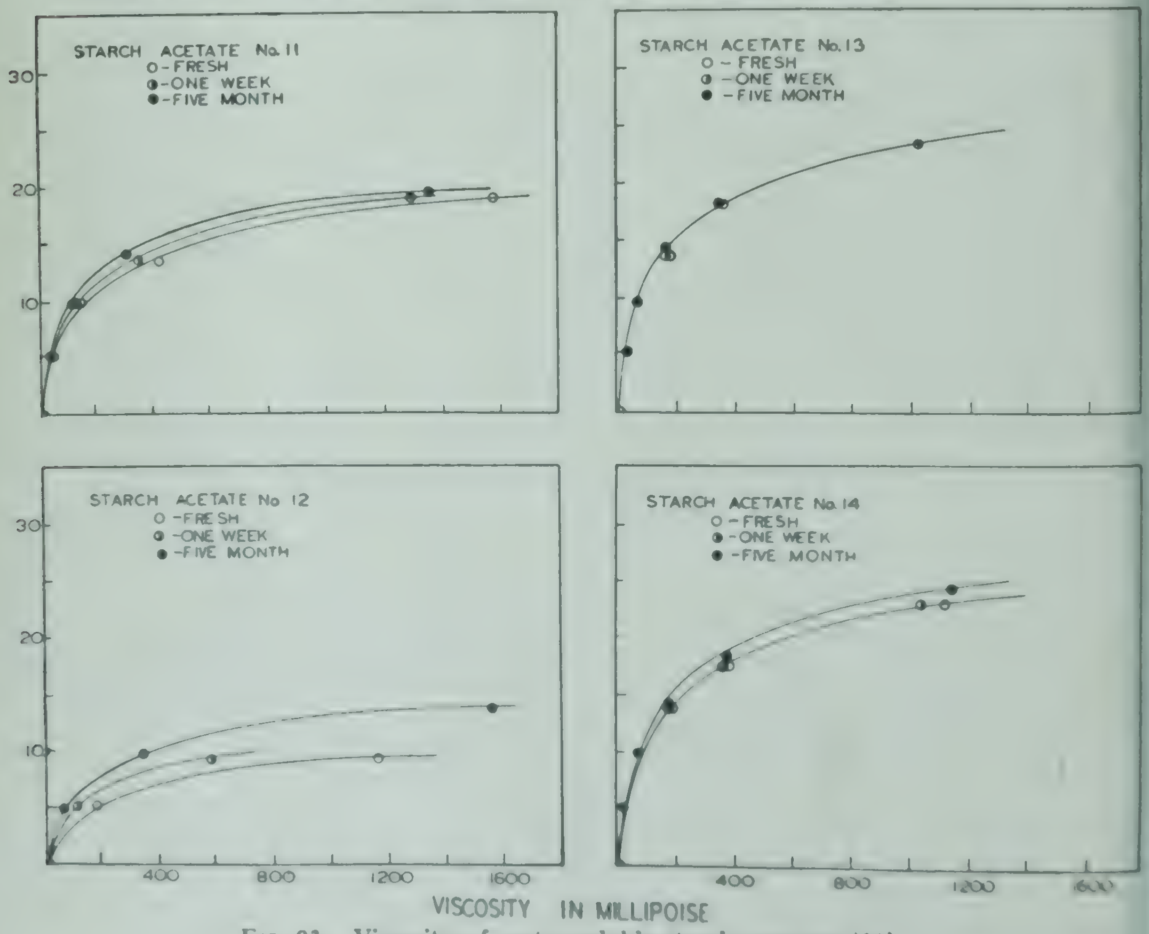


FIG. 83. Viscosity of water-soluble starch acetates (31).

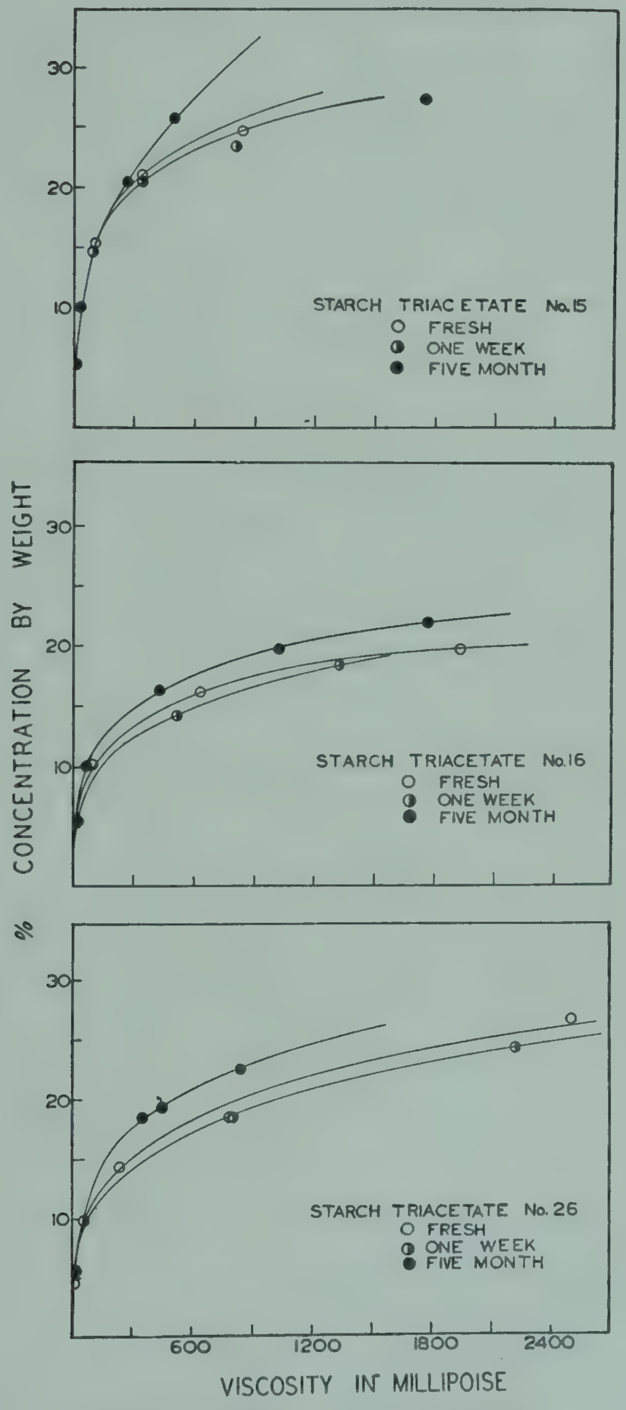


FIG. 84. Viscosity of starch triacetate.

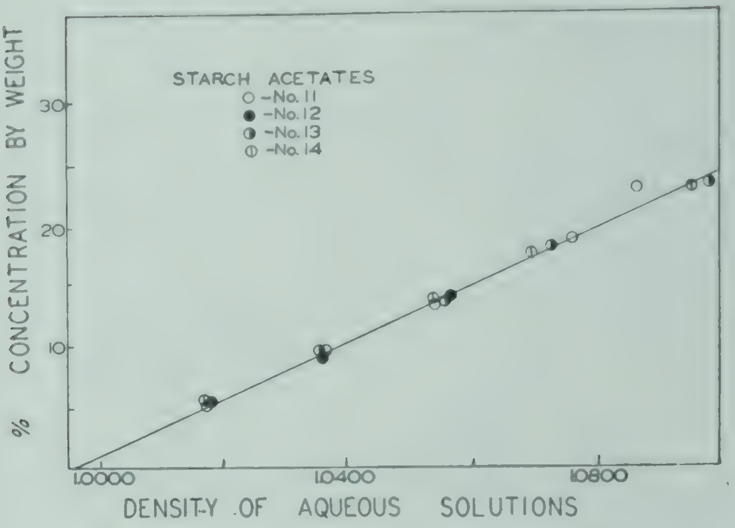


Fig. 85. Density of solutions of water-soluble starch acetates

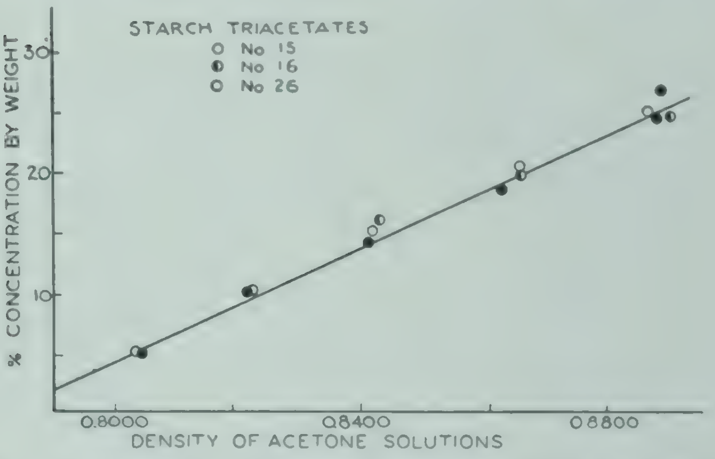


Fig. 86. Density of solutions of starch triacetate in acetone

where η is the viscosity in millipoise, d is the density of the solution, t is the time of flow in seconds, and A and B are constants peculiar to the viscosimeter.

The concentration of the solutions was measured by heating weighed portions of the solutions to constant weight in an oven. The density was measured with a 25 ml. pycnometer. Table XX contains these results. Figs. 83 and 84 give viscosity data and Figs. 85 and 86 density data. The plots of the density against concentration are linear.

As in the case of commercial cellulose acetates and nitrates, the blending of similar acetyl derivatives of starch of known viscosities to prepare a starch acetate of a desired viscosity is possible.

The solubility of the acetyl derivatives of starch in a large number of solvents has been determined by agitating an excess of the ester with 50 ml. of solvent in a sealed bottle in a thermostat at 25°C. for 24 hours. Those solvents which dissolve commercial cellulose acetates or cellulose nitrates also dissolve the acetyl derivatives of starch having an acetyl value greater than 40%. The derivatives having an acetyl value less than 15% are insoluble in organic solvents. Water does not dissolve the acetates possessing an acetyl value of more than 40%, while those of less than 15% dissolve to a slight extent at 25°C.

Some generalizations relative to decomposition temperature, specific gravity, optical rotation, viscosity, and solubility, are as follows:

1. The greater the degree of acetylation, the lower is the decomposition temperature of the ester. The esters of high viscosity have a higher decomposition temperature than the corresponding esters of low viscosity.
2. The specific gravities of the acetyl derivatives of starch are less than that of the original starch.
3. The specific rotation of the esters of starch becomes less as the extent of acylation increases.
4. Mercuric iodide prevents molding of the water solutions of the derivatives of starch.
5. The viscosity of solutions of the acetyl derivatives of starch is less than that of solutions of esters of commercial cellulose. A decrease in the viscosity is noted after standing for 5 months, and the magnitude of this change is proportional to the original content of free acid of the solution.
6. The viscosities of the triacetyl derivatives of starch are additive; so that esters of a given viscosity may be prepared.
7. The density of the acetyl derivatives of starch solutions is a linear function of the concentration.
8. The triacetates are not soluble in water, while the water-soluble esters are not soluble in organic solvents.
9. In the case of the triacetates, compounds containing keto groups, ester linkages, halogens as chlorine (except CCl_4), cyclic oxygen compounds, aromatic hydrocarbons and some of their functional derivatives, glycol ethers and their derivatives, and nitro paraffins are good solvents.
10. Ethers, aliphatic alcohols, aliphatic hydrocarbons, and polyhydric alcohols all tend to be poor solvents for the esters of starch.

Starch triacetates have been studied, furthermore, with respect to solubility in plasticizers, compatibility, and plasticizer retention. Two esters were used, a sample of low viscosity with an acetyl value of 41.0% and a sample of high viscosity with an acetyl value of 41.6%. The solubility of starch triacetates in plasticizers is included at 25°C., 100°C., and 180°C.,

TABLE XXI

Solubility and Compatibility of Starch Triacetate with Plasticizers

S = soluble; M = partially soluble; I = insoluble; C = compatible; P = compatible, but cracked; N = non-compatible; H = high viscosity; L = low viscosity.

Plasticizer	Solubility						Compatibility			
	25°C.		100°C.		180°C.		1 wk.		1 mo.	
	L	H	L	H	L	H	L	H	L	H
None							P	C	P	C
1. Dimethyl phthalate	I	I	S	S	S	S	C	C	C	C
2. Diethyl phthalate	I	I	S	S	S	S	C	C	C	C
3. Di-*n*-propyl phthalate	I	I	I	I	S	S	P	C	P	C
4. Diisopropyl phthalate	I	I	I	I	S	S	P	C	P	P
5. Dibutyl phthalate	I	I	I	I	S	S	P	N	P	N
6. Diamyl phthalate	I	I	I	I	M	M	P	N	P	N
7. Dioctyl phthalate	I	I	I	I	S	S	P	N	P	N
8. Dimethoxyethyl phthalate	I	I	S	S	S	S	C	P	C	P
9. Diethoxyethyl phthalate	I	I	S	S	S	S	P	C	P	C
10. Diphenyl phthalate	I	I	I	I	S	S	P	N	P	N
11. Dibutoxyethyl phthalate	I	I	I	I	S	S	N	C	N	C
12. Benzyl phthalate	I	I	S	S	S	S	P	C	P	P
13. Dimethyl sebacate	I	I	S	S	S	S	N	C	N	C
14. Diethyl sebacate	I	I	I	I	S	S	N	N	N	N
15. Dibutyl sebacate	I	I	I	I	S	S	N	N	N	N
16. Ditetrahydro furfuryl sebacate	I	I	I	I	S	S	N	P	N	P
17. Monotetrahydro furfuryl monobenzyl sebacate	I	I	I	I	S	S	C	C	N	C
18. Dimethoxyethyl adipate	I	I	S	S	S	S	C	P	C	P
19. Dicyclohexyl adipate	I	I	I	I	S	S	P	N	P	N
20. Butyl cellosolve adipate	I	I	I	I	S	S	N	P	N	P
21. Methyl-*o*-benzoyl benzoate	I	I	I	I	S	S	P	C	P	C
22. Ethyl-*o*-benzoyl benzoate	I	I	I	S	S	S	C	C	C	C
23. Benzyl benzoate	I	I	S	S	S	S	P	C	N	P
24. Diethyl tartrate	I	I	S	S	S	S	C	C	C	P
25. Dibutyl tartrate	I	I	S	S	S	S	C	C	C	C
26. Triacetin	S	S	S	S	S	S	P	C	P	C
27. Triproprionin	I	I	S	S	S	S	C	C	C	P
28. Tributyrin	I	I	S	S	S	S	P	P	N	P
29. Ethylene glycol diacetate	S	S	S	S	S	S	P	C	P	C
30. Triethylene diacetate	S	S	S	S	S	S	C	C	C	P
31. Tetrahydro β-naphthol acetate	I	I	I	I	S	S	N	N	N	N
32. Pentaerythritol tetraacetate	I	I	S	S	S	S	P	P	P	P
33. Glucose pentaacetate	I	I	I	I	S	S	P	P	P	P
34. Sucrose octaacetate	I	I	S	S	S	S	P	P	P	P
35. Ethylene dipropionate	S	S	S	S	S	S	C	C	C	C
36. Diethylene glycol dipropionate	S	S	S	S	S	S	C	C	C	C
37. Ethylene dibutyrate	I	I	S	S	S	S	C	P	C	P
38. Ethyl succinate	S	S	S	S	S	S	C	C	C	P

TABLE XXI (*Continued*)

Plasticizer	Solubility						Compatibility			
	25°C.		100°C.		180°C.		1 wk.		1 mo.	
	L	H	L	H	L	H	L	H	L	H
39. Lauryl salicylate	I	I	I	I	I	I				
40. Cyclohexyl laurate	I	I	I	I	M	M	N	N	N	N
41. Chlorinated methyl laurate	I	I	I	I	S	S	P	N	P	N
42. 2-Ethylhexyl laurate	I	I	I	I	I	I				
43. Ethyl crotonate	I	I	I	I	S	S	P	P	P	P
44. Butyl crotonate	I	I	I	I	S	S	P	P	P	P
45. Tributyl citrate	I	I	S	S	S	S	P	C	P	C
46. Acetyl triethyl citrate	I	I	S	S	S	S	P	C	P	C
47. Acetyl tributyl citrate	I	I	I	I	M	M	N	N	N	N
48. Butyl acetyl ricinoleate	I	I	I	I	M	M	N	N	N	N
49. Butyl stearate	I	I	I	I	M	M	N	N	N	N
50. Triethyl phosphate	I	I	S	S	S	S	C	C	C	C
51. Tributyl phosphate	I	I	S	S	S	S	P	C	P	C
52. Tricresyl phosphate	I	I	I	I	S	S	P	N	P	N
53. Triphenyl phosphate	I	I	S	S	S	S	P	C	P	C
54. Plasticizer 2 (Dow)	I	I	I	I	S	S	N	P	N	P
55. Plasticizer 5 (Dow)	I	I	I	I	S	S	P	P	P	P
56. Plasticizer 6 (Dow)	I	I	I	I	S	S	P	P	P	P
57. Plasticizer 7 (Dow)	I	I	I	I	S	S	N	P	N	P
58. Plasticizer 9 (Dow)	I	I	I	I	M	M	P	P	P	P
59. Santicizer 8	I	I	S	S	S	S	P	C	P	C
60. Santicizer 9	I	I	I	I	S	S	P	N	P	N
61. Santicizer 10	I	I	I	I	S	S	P	N	P	N
62. Santicizer B-16	I	I	I	I	S	S	P	P	P	P
63. Santicizer E-15	I	I	S	S	S	S	P	P	P	P
64. Santicizer M-17	I	I	S	S	S	S	P	C	P	C
65. Sanolite K	I	I	I	I	S	S	P	C	P	C
66. Sanolite MS	I	I	I	I	S	S	P	C	P	C
67. Sanolite MHP	I	I	I	I	S	S	P	C	P	C
68. Benzophenone	I	I	S	S	S	S	C	C	P	P
69. Acetamide	I	I	S	S	S	S	N	P	N	P
70. Acetanilide	I	I	I	I	S	S	P	C	P	C
71. Hercolyn	I	I	I	I	S	S	N	N	N	N
72. Abalyn	I	I	I	I	S	S	N	N	N	N
73. Sipalin AOM	I	I	I	I	S	S	P	N	P	N
74. Cyclonol	I	I	S	S	S	S	C	C	C	C
75. Triphenyl guanidine	I	I	I	I	S	S	P	P	P	P
76. Camphor	I	I	I	I	S	S	P	C	P	P
77. *N,N'*-Diethyl-*N,N'*-diphenylurea	I	I	S	S	S	S	P	C	P	C
78. Paraplex 5-B	I	I	I	I	S	S	N	N	N	N
79. Paraplex RG-2	I	I	I	I	S	S	P	N	P	N
80. Paraplex G-20	I	I	I	I	S	S	P	N	N	N
81. Flexol-Plasticizer 3-GH	I	I	I	I	S	S	P	C	P	C
82. Flexol-Plasticizer 3-GO	I	I	I	I	S	S	N	N	N	N

TABLE XXI (*Continued*)

Plasticizer	Solubility						Compatibility			
	25°C.		100°C.		180°C.		1 wk.		1 mo.	
	L	H	L	H	L	H	L	H	L	H
83. Butyl acetal	I	I	I	I	I	I				
84. Ethyl acetanilide	I	I	S	S	S	S	P	C	P	P
85. Ethyl-*p*-toluenesulfonate	I	I	S	S	S	S	P	P	P	P
86. Diphenyl ether	I	I	I	I	M	M	P	C	P	N
87. Polychlor naphthalene, 93°	I	I	I	I	I	I				
88. Polychlor naphthalene, 123°	I	I	I	I	I	I				
89. Polychlor naphthalene, 135°	I	I	I	I	I	I				
90. Plasticizer E-30	I	I	I	I	S	S	N	N	N	N
91. Plasticizer E-40	I	I	I	I	S	S	N	N	N	N
92. Plasticizer E-50	I	I	I	I	S	S	N	N	N	N
93. Plasticizer E-60	I	I	I	I	S	S	P	N	P	N
94. Aroclor 1242	I	I	I	I	S	S	P	P	P	P
95. Aroclor 1248	I	I	I	I	S	S	P	C	P	P
96. Aroclor 1254	I	I	I	I	S	S	P	P	P	P
97. Aroclor 1260	I	I	I	I	S	S	N	N	N	N
98. Soybean oil	I	I	I	I	I	I				
99. Linseed oil	I	I	I	I	I	I				
100. Cottonseed oil	I	I	I	I	I	I				
101. Castor oil	I	I	I	I	I	I				
102. Soyapole 75	I	I	I	I	I	I				
103. Ditetrahydro furfuryl maleate	I	I	S	S	S	S	C	C	C	C
104. Carbowax 1500	I	I	M	M	S	S	P	C	P	C
105. Carbowax 4000	I	I	I	I	M	M	N	N	N	N

1 g. of the starch triacetate and 10 g. of the plasticizer being used (*cf.* Table XXI). Corresponding studies of cellulose acetates have been reported by Fordyce and Meyer (69). Only plasticizers which dissolve starch triacetate at 180°C. are included. The solubility of the triacetyl derivatives of starch in plasticizers correlates very closely with the solubility data for commercial cellulose acetates or nitrates.

Films of acetyl esters of starch, obtained by the method of Reinhart and Kline for films of cellulose esters, were made. The triacetyl derivative of starch, plasticizer, and chloroform were mixed in proportions that gave 7 g. of the triester and plasticizer to 100 g. of chloroform, the ratio of starch triester to plasticizer being 9:1. The films were cast on cellophane in Petri dishes (100 × 15 mm.). The results are given in Table XXI. The films, observed at the end of 1 week and 1 month are reported as compatible, compatible but cracked, and non-compatible.

Retention of plasticizer by triacetyl starch films upon heating and leaching with water was measured for comparison with the work of Fordyce and Meyer on cellulose acetates (69). All films were heated under a pressure of about 29 inches at 35°C.

The loss of plasticizer by leaching with water was carried out by placing each film in an individual beaker in a thermostat and leaching at 40°C. for 24 hours. The water in the beaker was changed at frequent and regular intervals. The films were dried to a constant weight under reduced pressure, and the loss by leaching calculated. The films as formed were clear, but, after leaching, they became opaque. Table XXII records retention studies of plasticizers.

Some generalizations concerning solubility in plasticizers, compatability, and retention of plasticizer are as follows:

1. Naturally occurring vegetable oils do not dissolve the triacetyl esters of starch.

2. Esters of low molecular weight are good solvents, whereas esters of high molecular weight are poor solvents, needing higher temperatures for complete solution.

3. In the cases in which very large viscosities are found to occur in a plasticizer, the rate of solution is impeded.

4. The loss of weight by heating is higher, in general, for triacetyl starch films than for cellulose acetate films.

5. The loss of weight by leaching is also greater for triacetyl starch films than for cellulose acetate films.

6. Triacetyl starch films become opaque and brittle when leached.

The triacetyl derivative of "amylose" gives relatively strong films, whereas the corresponding ester of "amylopectin" gives relatively weak films (160a). This indicates that good film-forming properties are dependent upon the presence of certain structural units of the string-type molecule. Acetyl derivatives of whole starch give molded products which are brittle, clear, and transparent, whereas the comparable derivatives of "amylose" may be molded into strong and tough plastics (170). The acetyl derivative of starch fraction A of Schoch (221) forms films of high tensile strength and good extensibility, but the corresponding derivative of fraction B does not although both give clear, transparent, and lustrous films (257).

The x-ray analysis indicates that stretching produces orientation in films of acetylated "amylose," but the effect is much less pronounced in comparable films of "amylopectin" (260). The former are more linear than the latter and thus more susceptible to orientation by stretching (260). The effect of plasticizer on stress-strain properties has been noted (190).

d. Estimation of Acetyl Value. The estimation of acetyl value, by an exchange reaction of an acetylated starch with methanol in the presence of

TABLE XXII

Retention of Plasticizer by Starch Acetate Films

Plasticizer	Viscosity of ester	Original content of plasticizer	Loss in heating	Loss on leaching
		per cent	per cent	per cent
1. None	Low	00.00	5.28	14.96
2. None	High	00.00	6.89	13.45
3. Dimethyl phthalate	Low	10.60	9.80	11.95
4. Dimethyl phthalate	High	8.64	7.72	14.09
5. Diethyl phthalate	Low	9.46	5.16	11.62
6. Diethyl phthalate	High	8.85	7.82	13.12
7. Di-*n*-propyl-phthalate	Low	9.67	5.53	10.04
8. Di-*n*-propyl phthalate	High	7.82	5.24	10.16
9. Diisopropyl phthalate	Low	9.62	4.43	12.63
10. Diisopropyl phthalate	High	8.52	4.24	9.66
11. Dibutyl phthalate	Low	7.64	4.30	11.20
12. Dibutyl phthalate	High	12.98	9.97	10.32
13. Diamyl phthalate	Low	11.62	5.46	10.28
14. Diamyl phthalate	High	12.11	5.94	8.21
15. Diphenyl phthalate	Low	9.48	5.59	11.68
16. Diphenyl phthalate	High	8.89	6.67	9.78
17. Dibenzyl phthalate	Low	10.79	6.57	9.65
18. Dibenzyl phthalate	High	8.82	5.51	10.28
19. Dimethoxyethyl phthalate	Low	11.32	7.20	12.52
20. Dimethoxyethyl phthalate	High	9.78	6.84	12.02
21. Diethoxyethyl phthalate	Low	9.85	7.21	10.71
22. Diethoxyethyl phthalate	High	11.90	8.02	10.26
23. Dibutoxyethyl phthalate	Low	11.53	3.59	9.35
24. Dibutoxyethyl phthalate	High	12.18	4.47	8.88
25. Triethyl phosphate	Low	9.39	6.20	14.03
26. Triethyl phosphate	High	9.35	8.00	15.19
27. Triphenyl phosphate	Low	8.74	4.21	10.52
28. Triphenyl phosphate	High	10.74	5.32	11.53
29. Tributyl phosphate	Low	17.79	3.50	5.44
30. Tributyl phosphate	High	11.38	8.11	9.12
31. Tricresyl phosphate	Low	10.95	4.18	11.90
32. Tricresyl phosphate	High	10.22	3.62	8.52
33. Tributyl citrate	Low	8.61	4.03	9.21
34. Tributyl citrate	High	11.99	5.23	8.23
35. Acetyl triethyl citrate	Low	9.84	4.62	9.65
36. Acetyl triethyl citrate	High	8.70	4.04	9.37
37. Dibutyl tartrate	Low	9.44	3.88	11.20
38. Dibutyl tartrate	High	8.62	7.27	15.40
39. Diethyl tartrate	Low	10.63	3.62	17.81
40. Diethyl tartrate	High	10.93	7.14	11.35
41. Methyl-*o*-benzyl benzoate	Low	9.41	6.85	15.58
42. Methyl-*o*-benzyl benzoate	High	10.55	6.38	14.48
43. Ethyl-*o*-benzyl benzoate	Low	9.03	4.24	14.17
44. Ethyl-*o*-benzyl benzoate	High	9.59	[illegible]	15.88
45. Triphenyl guanidine	Low	10.18	3.09	12.24
46. Triphenyl guanidine	High	10.79	6.22	11.98
47. Triacetin	Low	12.01	7.03	17.50
48. Triacetin	High	11.72	6.20	21.40

p-toluenesulfonic acid, was used by Freudenberg and Harder in 1923 (77). By a modification of the Eberstadt method for the determination of acetyl in cellulose acetate, an acetyl derivative of starch is swollen with warm alcohol (75%) and then saponified, below 35°C., for 24 to 48 hours with aqueous alkali (0.25 *N*, initial concentration) (87). The excess alkali is back-titrated carefully with standard acid (87).

By one procedure, 1 g. of an acetyl derivative of starch is suspended in 50 ml. of carbon dioxide-free water and titrated with 0.1 *N* sodium hydroxide solution until barely alkaline to phenolphthalein. This is a measure of the absorbed acid. One then adds 25 ml. of 0.5 *N* sodium hydroxide, heats the suspension with stirring, and boils the solution for 5 minutes. The excess base is then titrated with 0.5 *N* sulfuric acid and the acetyl value of the ester calculated from the amound of base required for the saponification (30).

By another procedure, 1 g. of the ester is placed in a glass stoppered flask, covered with 10 ml. of methanol, allowed to stand for an hour, and then treated with 20 ml. of 1 *N* sodium hydroxide solution (169). This mixture is allowed to stand at room temperature, with occasional agitation, for 12 hours and then titrated with 0.5 *N* hydrochloric acid. The amount of alkali required for the saponification is used to calculate the acetyl value (169).

By use of an exchange reaction, a 0.2 to 0.3 g. sample of the finely divided ester, previously dried under reduced pressure over phosphorus pentoxide, is placed in 20 ml. of anhydrous methanol in a reaction flask. Five to 10 ml. of 0.2 *N* sodium methoxide in methanol is then added slowly through a dropping funnel. The temperature is then raised to maintain the distillation of about 35 drops per minute into a receiver packed in ice. When the volume is reduced to about 5 ml., 20 ml. of anhydrous methanol is again added through the dropping funnel. Two additional portions of anhydrous methanol, 10 ml. each, are added and distilled. The distillate is then saponified and the excess alkali titrated (258). A modification of the procedure makes use of *p*-toluenesulfonic acid as the catalyst (42).

e. Uses of Acylated Starches. The acylated starches may be used in (*a*) the preparation of coating and "livering" compositions (157), (*b*) the synthesis of ethers through exchange reactions (99, 100), and (*c*) the production of chlorinated acylated starches by treatment with phosphorus pentachloride in toluene (185).

3. Alkyl Derivatives of Starch

a. Historical Review. The first recorded interest in the ethers of starch is disclosed in a patent, which was issued in 1912 (146). Part of the work since then on the production of ethers of starch has been activated entirely by structural considerations of the starch molecule (76, 78, 112).

In 1920, West published a paper in which he considered the raw materials and the procedure for the production of the ethers of starch (255). He characterized some of the ethers he obtained. In the same year Bayer obtained a patent for the manufacture of the hydroxy ethers by the reaction of the alkene oxides on starch (9). A similar patent was granted to I. G. Farbenindustrie. Dreyfus, in 1920, also obtained a patent for the production of hydroxy ethers of starch by use of such reagents as ethylene chloride, ethylene chlorohydrin, epichlorohydrin, and similar chemicals in the presence of appropriate condensing agents (53).

In 1921, Gomberg treated different starches with benzyl chloride in the presence of sodium hydroxide (88). He observed that the colloidal and paste properties of these derivatives might warrant their use as industrial products. Other patents were secured by Lilienfeld during 1921 for the production of the ethers by treatment of starch with alkyl halides under appropriate conditions (145).

Gault (1923) treated starch with lauryl chloride in the presence of pyridine and toluene at 100°C. for 2 hours, and obtained an 80% yield of dilauryl starch upon precipitation with alcohol (84). He reported that this ether is soluble in benzene and chloroform and that it is non-inflammable. During the same year, Tomecko and Adams reported that they had treated both corn and potato starch with allyl bromide in the presence of aqueous alkali (247). They obtained and characterized both a monoallyl corn starch and a monoallyl potato starch.

Helferich and Koester, in 1924, treated starch with chlorotriphenylmethane in pyridine to give the corresponding ether (102). This ether swells in organic solvents and is readily decomposed by water to regenerate the starch and give hydroxytriphenylmethane.

Schmid and Zentner, in 1928, described a procedure for the preparation of methylated starches by treating starch with diazomethane (219). During the next few years various workers obtained patents for methods of producing the alkyl ethers of starch. Lilienfeld, for example, obtained additional patents in 1929 and 1930 (147).

In 1934, Maksorov and Andrianov reported on the benzyl ethers of different starches and compared the properties of these derivatives (155).

Ziese, in 1934 and 1935, treated starch with ethylene oxide in alkaline solution and obtained the corresponding hydroxy derivatives of starch (270). During the same years, patents were assigned to du Pont for the production of ethers of starch by use of sodium alkyl sulfates as the alkylating agents (54). The patents claim that a variety of products is obtainable by certain variations in the experimental conditions.

Pancirolli (1937) treated alkali starch with *p*-aminobenzyl chloride (183). Such a compound may be utilized in diazotization and coupling reactions.

Much of the work on the methyl derivatives of starch has been by those who were interested in the structure of the molecule (76, 78, 112), but the preparation of the higher derivatives has usually been inspired by a quest for important new commercial products.

Alkylating agents which have been used in the synthesis of ethers of starch include: alkyl halides (145, 147), allyl bromide (247), allyl chloride, benzyl chloride (88, 155), chlorotriphenylmethane (102), diazomethane (218, 219), epichlorohydrin (53), ethylene chloride (53), ethylene chlorohydrin (53), ethylene oxide (9, 63), lauryl chloride (84), methyl sulfate (120), *p*-nitrobenzyl chloride (183), and sodium alkyl sulfates (54).

Condensing agents which have been reported for the alkylation of starch include: aqueous alkali (147), 30% aqueous sodium hydroxide (100), calcium chloride (62, 147), pyridine (102), pyridine in toluene (84), and a tertiary-amine base (147).

b. Preparation of Alkyl Starches. The patent literature covers the production of various ethers by use of an alkylating agent in caustic (62, 83, 145, 146). Directions for the preparation of the methyl derivatives of starch are given by Karrer (126) and others (99, 100). The methylated starches have been prepared from both raw starch (120) and triacetyl starch (99, (100). Raw starch of both rice and potato, after 24 successive methylations with methyl sulfate and caustic, gave only a 25% yield of trimethyl starch (120). A (1) dimethylstarch (99, 100) and a (2) trimethylstarch may be prepared in satisfactory yields (99, 100). The ethyl derivative may be obtained by the directions outlined in the patent of Lilienfeld (146).

Consideration has been given to both the raw material and the procedure for the production of ethers of starch. Some of the ethers have been prepared and characterized (255). Patents cover the production of the ethers of starch by treatment of starch with alkyl halides in the presence of an appropriate condensing agent (145, 147). The treatment of starch with sodium alkyl sulfates under proper conditions results in the production of the corresponding ethers of starch (54). A variation in the experimental conditions results in a variation in the properties of the product of the reaction (54).

Powdered triacetyl starch (20 g.) is treated with 30% sodium hydroxide solution (224 ml.) and methyl sulfate (80 ml.), both added gradually and simultaneously with vigorous mechanical stirring. The temperature is maintained at 50°C. for 75 minutes then at 70°C. for another 75 minutes, and finally at 100°C. for 30 minutes. Mild alkalinity is maintained in the reaction flask throughout. The solution is then cooled, neutralized, filtered, and the solid residue extracted with boiling chloroform to give about an 80% yield of dimethyl starch (99, 100). The triacetyl starch was prepared from potato starch by the use of acetic acid and acetic anhydride in the presence of chlorine and sulfur dioxide (99, 100).

Powdered triacetyl starch (25 g.) is dissolved in acetone (250 ml.) and treated, simultaneously and gradually, with methyl sulfate (100 ml.) and 30% sodium hydroxide solution (280 ml.), while the reaction mixture is maintained at 56°C. The acetone is then distilled, the mixture maintained at 100°C. for 30 minutes, cooled, neutralized, and filtered. Both the filtrate and the residue are extracted with chloroform and the chloroform recovered to give a 96% yield of trimethyl starch (99, 100).

Similarly, the acetyl dextrins may be converted into methyl dextrins by dissolving in acetone and treating, slowly and simultaneously, with methyl sulfate and 30% aqueous sodium hydroxide at about 53°C. (99).

The acetyl derivative of α-amylodextrin, when dissolved in acetone and then treated, slowly and simultaneously, with methyl sulfate and 30% aqueous sodium hydroxide at 55°C., yields the methyl α-amylodextrin (99). The acetyl derivative of "amylose" may be deacetylated and methylated simultaneously by use of methyl sulfate and 30% aqueous sodium hydroxide in acetone at 55°C.

The acetyl derivative of α-amylodextrin (prepared by treating α-amylodextrin with acetic anhydride in pyridine) may be converted to the methyl ether also by solution in acetone and treatment with methyl sulfate and 30% aqueous solium hydroxide, slowly and simultaneously, at room temperature for 5 hours (99). A glycollic acid derivative of starch has been used also for the preparation of the corresponding methyl ether.

The ethyl ether of starch may be prepared by effecting ethylation of starch with ethyl sulfate in the presence of alkali. One hundred grams of starch (10% H_2O) is treated with 900 ml. of 10% sodium hydroxide solution in a large alkylation apparatus and then 200 ml. of 30% sodium hydroxide solution is added slowly with stirring. The mixture is heated to 60°C., 200 ml. of ethyl sulfate added dropwise with stirring, and the mixture then heated to 100°C. for an hour. The mixture is cooled, divided for convenience into two portions, and 300 g. of finely divided sodium hydroxide added to each portion. Each portion is then warmed up to 60°C. and treated over a period of 2 hours with 300 ml. of ethyl sulfate. The solutions are finally heated on a water bath at 100°C. for an hour, neutralized and filtered or filtered as is, then washed well and dried. The resulting product is soluble in organic solvents but insoluble in water (yield, 27.65%, 2.13 ethoxy groups per anhydroglucose unit).

The patent literature discloses the propyl ether of starch (269), which may be synthesized by heating together in a stirred or rocking autoclave, at 140°C. for 24 hours, 30 g. of corn starch (10 to 15% H_2O), 60 g. of solid sodium hydroxide, 450 g. of a 40% solution of sodium hydroxide, and 277 g. of *n*-propyl chloride (49, 50a). The reaction mixture is subjected to steam distillation to remove the lower alcohols and lower ethers (50a). The grey, solid residue is extracted with glacial acetic acid, filtered, and the filtrate poured, with efficient agitation, into an excess of cold water. The white product which floats to the surface is filtered, washed with sodium carbonate, washed with distilled water, and dried (yield, 30 g.).

The butyl ether of starch is prepared by heating, in a rocking or stirring type autoclave at 165°C. for 24 hours, 15 g. of corn starch, 250 g. of 40% sodium hydroxide, and 160 g. of *n*-butyl chloride (50a). The excess butyl chloride, the butyl alcohol, and the butyl ether are then removed by steam distillation (50a). Upon cooling, a compact, brittle solid is obtained. This is dissolved in glacial acetic acid and the solution poured into an excess of cold water to precipitate the ether. This purification step may be repeated. A white, fluffy product (18.4 g.) is obtained, which is soluble in some organic solvents but insoluble in water (50a).

The treatment of starch with lauryl chloride in the presence of pyridine and toluene at 100°C. for 2 hours, and subsequent precipitation with alcohol, gives a dilauryl derivative (80% yield). This ether is soluble in benzene and in chloroform and is non-inflammable (84).

The treatment of starch with chlorotriphenylmethane in pyridine yields the corresponding ether, which swells in organic solvents and is readily decomposed by water to give starch and hydroxytriphenylmethane (102).

The hydroxy derivatives of starch are prepared readily by the reaction between starch and alkene oxides, particularly ethylene oxide, in the presence of aqueous alkali (9, 63, 150). A different procedure condenses starch with chemicals such as ethylene chloride, ethylene chlorohydrin, epichlorohydrin, and sodium chloroacetate, in the presence of appropriate condensing agents, to yield substituted ethers of starch (53, 151).

The monoallyl ether of both corn and potato starch is prepared by treatment of the respective starches with allyl bromide in the presence of aqueous alkali (247). Allyl starch is now produced by condensing allyl chloride with starch. The soluble allyl ether of starch has been prepared from starch acetate as well as by the direct allylation of starch (173, 174) and studied as a plastic material (188). According to patent claims, the cyanoethyl ethers have been obtained also (29). A number of mixed ethers of starch, which contain both allyl and alkyl groups, have been prepared (96).

The benzyl derivatives of starch are prepared from different starches by treatment of the starch under study with benzyl chloride in the presence of sodium hydroxide (88, 155). These derivatives have colloidal and paste properties which indicate certain commercial uses (88, 155). Benzylated starches vary with the source of the starch. These ethers are soluble in acetone, alcohol, and the aromatic hydrocarbons (155). The films and castings are brittle and glossy (155).

The treatment of alkali starch with compounds such as *p*-nitrobenzyl chloride yields derivatives which may in turn be reduced and diazotized and coupled to other compounds (183). Highly substituted, uniform starch ethers may be prepared at lower reaction temperatures and with shorter reaction times by preswelling the starch with formic acid (172, 180).

c. Properties of Alkyl Starches. The methyl derivative of starch is water-soluble and therefore must meet the competition of cheaper starch products. The ethyl derivative of starch can be made both as water-soluble and insoluble derivatives. One alkyl group per glucose residue produces solubility in water whereas two ethyl groups give a water-insoluble product. The latter product is more soluble in organic solvents.

TABLE XXIII

Solubility of Alkylated Starches

Solvent	Ethyl derivative	Propyl derivative	Butyl derivative
Acetone	Soluble	Soluble	Soluble
Toluene	Soluble	Soluble	Soluble
Amyl acetate	Soluble	Soluble	Soluble
Butanol	Soluble	Soluble	Soluble
Petroleum ether	Insoluble	Partially soluble	Partially soluble
Tetrachloroethane		Soluble	Soluble
Ethyl alcohol (95%)	Soluble	Soluble	Partially soluble

The propyl derivative of starch is definitely water-insoluble when two propyl groups per glucose unit have been introduced into the molecule. The butyl derivative of starch is even more like the non-polar type of organic compound. The solubility characteristics are given in Table XXIII.

These alkylated derivatives of starch are compatible with commonly used plasticizers such as dibutyl phthalate, tributyl phosphate, Ethox, Flexol, tributyl tritrate, and Hercolyn.

Because of the nature of the compounds, no accurate melting points have been obtained for these derivatives. The observable physical change can be described as a shrinking or softening. The points of softening (uncorrected) for the alkyl starches are as follows: for the ethyl derivative, 140-145° C.; for the propyl derivative, 105-110° C.; and for the butyl derivative, 73-76° C.

TABLE XXIV
Specific Gravity of Alkyl Starches

Derivative of starch	Specific gravity	Temperature
		°C.
Ethyl	1.14	27
Propyl	1.05	28
Butyl (kerosone used as liquid)	0.88	28

As might be expected, the specific gravity of the alkyl ethers of starch vary with the length of the alkyl group introduced (Table XXIV). The products have about the same specific gravity as the corresponding alkyl ethers of cellulose, and hence have a large spreading power in comparison with that of more dense materials such as the acetate or nitrate of starch or cellulose.

TABLE XXV
Relative Viscosity of Alkyl Starches

Derivative of starch	Time	Viscosity
	sec.	centipoises
Ethyl	586	2.92
Propyl	390	1.97
Butyl	210	1.06

The viscosity of these alkylated products varies inversely as the chain length of the alkyl group. The higher alkyl derivatives require more drastic means for their preparation, and this is reflected in the degradation which contributes to the low viscosity. Table XXV contains the viscosity in centipoises of these derivatives as determined in a 5% toluene solution with the Ostwald viscosimeter.

4. Nitric Acid Derivatives of Starch

a. Historical Review. The first attempt to prepare the nitric acid esters of starch appears to have been in 1833, when Braconnot treated starch with concentrated nitric acid and obtained a product which he termed xyloidine (24). He observed that textiles impregnated with xyloidine and then dried were found to retain their stiffness and impermeability even after treatment with boiling water. In 1844, Ballot described in detail the preparation of xyloidine and gave some consideration to its constitution (5).

Pelouze in 1846, examined xyloidine and pyroxylin critically and found that they differ with respect to both their chemical composition and physical properties (187). He considered, furthermore, the probable utilization of such derivatives of starch for military explosives.

In 1847, Kindt reported a microscopic study of the transformations which accompany the reaction of mixed acid (nitric and sulfuric acids) on starch (134). Payen (1847) verified the preparation of xyloidine by earlier workers, and observed that the starch granule consists of an interior and an outer portion (184). The inner portion, he observed, gives an intense blue coloration with iodine, whereas the outer portion does not. In 1849, Reinsch made a detailed report, which is available in the Columbia College of Pharmacy, on starch nitrate (201).

The regeneration of starch from xyloidine was reported by Béchamp in 1853 when he treated this ester with certain ferrous salts (10). In 1860, he described the nitric acid ester of both dextrin and starch (10). In the following year (1861) Ritter described the preparation of the nitrate of starch and discussed its explosive properties (204).

Béchamp, in 1862, obtained a nitric acid ester of starch (6.7% N) which was soluble in aqueous acetic acid but insoluble in the usual organic solvents (10). He obtained also a starch nitrate which was soluble in acetone, alcohol, and ether, and contained about 11% nitrogen. He decomposed all of these by the action of ferrous chloride to regenerate the starch and liberate nitric oxide. The rotatory power of the various nitrates of starch were observed and recorded.

Berthelot reported in 1876 the amount of heat evolved when starch is treated with concentrated nitric acid to give xyloidine (19). In 1885, he reported further studies on xyloidine.

The history of the preparation and properties of the nitric acid esters of starch was reviewed by Mühlhauser in 1892 (167). At this time he reported the preparation of esters that ranged in nitrogen content from 10.5 to 13.5%. He showed, furthermore, that these derivatives are not nitro compounds but true esters.

Will, in 1898, nitrated starch by two different methods to obtain products which contained from 13.9 to 14.04% nitrogen (261). These derivatives were soluble in both alcohol and ethyl acetate. They were comparatively stable at 50°C. but decomposed with explosive violence at 194°C. He considered these products to be the hexanitrates of starch.

In the following year (1899) Brown and Millar prepared nitrates of starch containing from 7.8 to 11.5% nitrogen (27). Determinations of molecular weight by lowering of the freezing point of acetic acid gave values around 987. When these derivatives were treated with ammonium sulfide, soluble starch was regenerated. Sapozhnikov, in 1903, reported the preparation of nitrates of starch (13.4% N) by two different methods (215). Molecular weight determinations by the ebulliometric method gave a value of 1845.

The nitric acid ester of starch was decomposed by Berl, in 1908, by treatment with alcoholic sodium hydroxide (18). The reaction mixture was acidified, and a product obtained whose osazone resembled that obtained from oxypyruvic acid. Traquair, in the following year (1909), published the results on the preparation and properties of the nitric acid esters of starch (248).

Four different varieties of starch were nitrated by Berl and Bütler in 1910 (15). They treated the starch with mixed acid and obtained products which contained from 12.86 to 13.85% nitrogen. These derivatives were studied with respect to solubility in alcohol and ether, hygroscopicity, ignition point, and viscosity. They observed that the viscosity of a 5% solution of cellulose nitrate is about 9000 times that of a corresponding solution of starch nitrate.

Cope, in 1917, reported comparative determinations of the nitrogen of starch nitrate by use of the nitrometer and the nitro method (40). In the same year (1917) Sadtler described in some detail the preparation and properties of nitrates of starch which contained from 12 to 13.3% nitrogen (206).

A patent on the production of starch nitrates was secured by Anchors in 1920 (1). In 1922, Kessler and Röhm presented a critical review of the nitration processes and the properties of the various nitric acid esters of starch (132). Another discussion of the nitrates of starch was published by Okada in 1927 (176).

In 1935, Berl and Kunze published a paper entitled *Zur Kenntnis der Stärkenitrate,* in which they considered the nitration of starch, the morphology of starch nitrate, and the viscosity of starch nitrate (17).

b. Preparation of Nitric Acid Esters of Starch. Starch was nitrated by Berl and Kunze under different conditions (17). They found that mixed acid gave a better product than did concentrated nitric acid, and a nitric-phosphoric acid mixture gave better results than did mixed acid. The phosphoric acid, in the latter case, functions much as the sulfuric acid in mixed acid and becomes fixed in the resulting product to the extent of 0.19% or more.

The esters obtained under these conditions contained 13.0 ± 0.1% nitrogen, whereas the theoretical vaue is 14.14%. These workers believe that values in the literature of about 14.10% nitrogen for nitrates of starch are erroneous, or else the starch was carefully fractionated and purified before nitration. Maximum nitration was effected in about 30 minutes.

According to Caesar and Goldfrank, starch is dried to a moisture content of less than 1%, suspended in an inert solvent such as chloroform or carbon tetrachloride, and then nitrated with nitrogen pentoxide, in the presence of a nitric acid binding agent such as sodium fluoride, at a temperature of 16 to 18°C. (33). The reaction mixture is filtered, washed with chloroform, slurried with cold water, filtered, washed with hot water, and stabilized by boiling with dilute ammonium hydroxide (33). The product (13.9 to 14% N) is stored in distilled water or 50% ethanol (33).

Solvent extraction has been used to separate the nitric acid esters of starch into fractions, which differ appreciably in their nitrogen content and in their physical properties (17, 196).

When completely dry starch is treated with 100% phosphoric acid at 0°C., there is no apparent action. Upon the addition of ice-cold nitric acid to this mixture, nitration sets in almost instantly, and the nitrogen content reaches a maximum in about 10 minutes. The resulting product is very stable and has a viscosity much higher than that of the derivative obtained from the action of either concentrated nitric acid or mixed acid on starch.

c. Properties of Nitric Acid Esters of Starch. The viscosity of the nitrates of starch is inversely proportional to the extent of the degradation of the molecule. Nitration with mixed acid gives a maximum viscosity at the end of about 1 hour. At the end of 12 hours the viscosity has decreased to about one-half of the maximum. Much less degradation is effected with the nitric-phosphoric acid mixture, as evidenced by the fact that the maximum viscosity of the reaction mixture is attained at the end of about 12 hours.

The viscosity of the nitrates of starch prepared in the presence of phosphoric acid was about 10 times as great as comparable derivatives prepared in the presence of sulfuric acid. These results check with similar studies made on the nitrates of cellulose.

The viscosity of a solution of the nitrates of starch with identical nitrogen content seems to be dependent upon (*a*) the composition of the mixed acid used in effecting nitration, (*b*) the grade of the starch used, (*c*) the time of nitration, (*d*) the temperature at which nitration is effected, and (*e*) other variables.

Among the variables may be mentioned pretreatment. The data indicate that the viscosity of the nitrated product of a pretreated starch is less than that of the comparable nitrate of the untreated starch. Decrease in viscosity definitely indicates a decrease in the molecular weight of the parent starch molecule. Qualitatively, moreover, the viscosity of the nitrates of starch increases with an increase in the grain size of the starch granule.

When starch is boiled under pressure, there is a decrease in viscosity to a minimum, but when this starch is nitrated there is an increase in viscosity. A value of 28,000 to 31,000, for example, is to be compared with a value of 48,000, as shown in Tables XXVI and XXVII.

The nitrates of starch are relatively unstable, but the product obtained from a nitric-phosphoric acid mixture on starch is much more stable than

TABLE XXVI

Analysis of Two Comparable Nitrates of Starch

Type of nitrate	Nitrogen	Viscosity at 20°C.
	per cent	*poises*
Amylopectin	11.82-12.05	28,000-31,000
Amylose	13.40-13.45	61-84

TABLE XXVII

Effect of Precooking under Pressure on Viscosity

Temperature	Pressure	Viscosity of nitrate
°C.	*atmospheres*	*poises*
90	0.715	48,000
100	1	38,000
110	1.4	37,140
120	2.0	34,760
130	2.7	28,100
150	4.7	1,600
160	6.1	42
170	7.8	14
180	10.0	6

that obtained from mixed acid. Since the phosphoric acid esters of starch are much more stable than the sulfuric acid esters, it is believed that the decomposition of nitrates may be initiated to some extent at least by the small amont of the sulfates or phosphates present. The relative stability of the nitrates of starch may be increased by (*a*) the use of purified starch, (*b*) thoroughly washing with hot water to remove traces of acid, and/or (*c*) treatment with aniline (175), alkaline solutions (175), carbonates (207), or methanol (17).

A critical comparison of the starch with the nitrated starch gave indication of two types of both starch and of nitrated starch; the bright hull which is termed *amylopectin nitrate* by these workers, and the dark nucleus which they called *amylose nitrate*. These products may be separated by grinding up the aqueous suspension and then permitting fractional sedimentation to take place. The denser fraction, *amylose nitrate*, has the higher nitrogen content. The *amylose nitrate* dissolves in acetone to give a clear solution, whereas the *amylopectin nitrate* tends to form a slimy suspension.

5. Phosphoric and Sulfuric Acid Derivatives of Starch. Blondeau, in 1843, treated starch with concentrated sulfuric acid and isolated and analyzed two lead salts of the derivative (21). He concluded that the reaction gives two starch bisulfates, or as he termed them, starch sulfonic acids. In 1845, Fehling prepared and analyzed a number of salts of starch bisulfate (66). In the same year Kalinowsky prepared and analyzed the calcium salt of starch bisulfate (125).

In 1883, Liechti reported the preparation of the sulfuric-oleic acid derivative of both dextrin and starch (144). He described the products as being water-soluble, as forming metallic salts, and as hydrolyzing to yield the corresponding carbohydrate and oleic and sulfuric acids.

Hoenig, in 1885, reported the treatment of starch with concentrated sulfuric acid to give a derivative which he isolated and described as the barium, calcium, and lead salts (113). In the following year he published another paper on the reaction of concentrated sulfuric acid on starch, and at this time gave some consideration to the structure of the starch molecule (112).

The treatment of starch with concentrated sulfuric acid effects both degradation and esterification (34). The former reaction is decreased and the latter increased by the use of an inert diluent such as chloroform, a relatively inert base such as pyridine, chlorosulfonic acid in place of concentrated sulfuric acid, and a careful control of the temperature. Vacuum-dried starch was added slowly to a chlorosulfonic acid in a mixture of chloroform and pyridine by Tamba in 1923 (241). The product was isolated as its potassium salt and characterized as the salt of an amylodisulfuric acid. This derivative showed no reducing action and gave no coloration with iodine. The treatment of alkali starch with *p*-toluenesulfonic acid was effected by Fukushima and Takamatsu in 1929 (83). This derivative was thought to contain one acid residue per glucose unit.

Starch suspended in chloroform was treated with phosphorus oxychloride in the presence of calcium carbonate by Kerb in 1919 (130). He isolated and identified both a calcium and a lead salt of a phosphoric acid ester of a degraded starch.

Samec, in 1927, treated phosphorus-free "amylose" with phosphorus oxychloride in pyridine (209). He obtained a phosphorylated derivative which he believed to be an "amylopectin." The first patent to cover derivatives of this general type was obtained by Traube in 1928 (249).

The phosphoric acid esters of starch occur in nature (208). The "amylopectin" of potato starch, for example, contains about 0.07 to 0.09% of phosphorus, which is probably present in the form of an ester in the ratio of about one phosphoric acid residue for every 212 to 273 anhydroglucose units (208). When this "amylopectin" is hydrolyzed and the products separated by electrodialysis, considerable amount of glucose-6-phosphate are obtained (191). This suggests that the ester is principally of the 6- or primary type. The separation of this "amylopectin" by electrodialysis is attributed o the strong anodic migration of the molecule as a consequence of the phosphoric acid residue (39).

The phosphoric acid ester linkage in starch is hydrolyzed very slowly by acid (10), more readily by prolonged heating with or without acid (214), and still more readily by alkali (213) or the use of amylophosphorylases.

6. Carbon Disulfide Derivatives of Starch. Cross, in 1907, saturated dry starch with carbon disulfide, then stirred in powdered sodium hydroxide, dissolved the product in water, acidified the mixture, and added iodine solution to precipitate a starch dixanthate (45).

In 1911, Ost treated starch with a 5 to 20% solution of sodium hydroxide, then with carbon disulfide, and allowed the mixture to stand for 24 hours (177). The aged mixture was poured into alcohol to precipitate the sodium starch xanthate. Analysis indicated 2 equivalents of sodium and 2 equivalents of sulfur per glucose unit. Ost observed, furthermore, that this sodium starch xanthate undergoes a progressive degradation with an accompanying decrease in viscosity. He reported, however, that the rate of degradation is somewhat slower than in the case of cellulose xanthate.

A patent was secured by Stern in 1922 to cover the production of the starch xanthates for use as an adhesive for wood veneers (236). According to his procedure, 15 kg. of sodium hydroxide is dissolved in 75 kg. of water and added slowly with agitation to 200 kg. of starch which has been thoroughly stirred with an equal volume of water at 60°C. Into the resulting mixture is stirred 7.5 kg. of carbon disulfide, accompanied by external cooling.

A study of the preparation and properties of the xanthates of starch was published in 1925 (264), a patent issued in 1932 covers their reaction with diazonium salts to give an organic dithiocarbonate (116), and a patent issued in 1933 covers their use as textile sizes (56).

The xanthates of starch undergo the same general types of reactions as do the corresponding xanthates of cellulose (177). They undergo progressive degradation with an accompanying increase in viscosity (177). This is accompanied by a decrease in the amount of bound carbon disulfide. The xanthates of starch are decomposed, with the regeneration of carbon disulfide and starch, by treatment with glacial acetic acid, heat, or mineral acid.

7. The Halogen Derivatives of Starch. In 1929 Bergmann and Ludewig presented evidence which indicates that bromine in the presence of potassium bromide and iodine in the presence of potassium iodide undergo reaction with certain starches or starch derivatives in some manner which involves a chemical union (14). They attributed the reaction to the presence of the oxygen bridges in the starch molecule (14).

More recently Barham and Thomson have studied the chlorination of both raw starch and of pretreated starch (244). The reaction is carried out in an autoclave at a temperature of 70°C., for eight or more hours, with a tenfold excess of chlorine (244). The principal products, under these conditions, are a mono- and a dichloro derivative of a partially degraded starch (244).

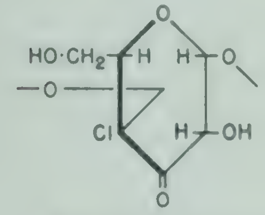
The dichloro derivative is prepared by treating 29.66 g. of a vacuum-oven-dried corn starch in an autoclave with 525 g. of chlorine for 36 hours at 70°C. (244).

The trichloro derivative is prepared by treating 10 g. of oven dried starch (0.38% water) in an autoclave with 580 g. of chlorine for 24 hours at 70°C. to give 13.12 g. of a lemon-yellow granular product (40.86% Cl) (244).

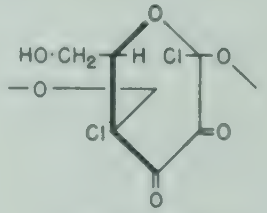
The tetrachloro derivative is obtained directly from the dichloro derivative by withdrawing the vapors from the autoclave and then recharging with 525 g. of chlorine and heating at 99 to 104°C. for 24 hours (244).

The hexachloro derivative is obtained by treating 24.39 g. of dry corn starch with 520 g. of chlorine in an autoclave at 80°C. for 24 hours to give first the dichloro derivative (244). The gases are then bled off, 520 g. of chlorine added, and heating continued at 95 to 102°C. for 24 hours to give the tetrachloro derivative. The gases are bled off again, 520 g. of chlorine added, and heating continued at 109 to 111°C. for 24 hours. The product is 43.5 g. of a brown, thick, viscous liquid (52.88% Cl) (249).

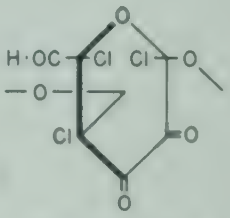
The structural units involved in these successive stages, as postulated by Barham and Thomson, are (244):



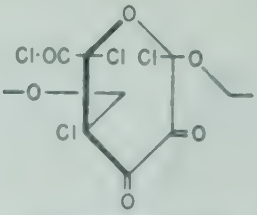
Monochloro Unit



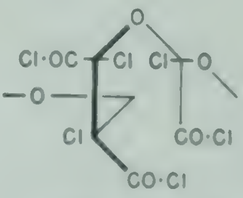
Dichloro Unit



Trichloro Unit



Tetrachloro Unit



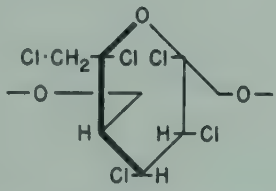
Hexachloro Unit

The use of phosphorus pentachloride, in the presence of chloroform or phosphorus oxycloride, for the synthesis of halogen derivatives of starch has been studied by Barham *et al.* (7).

An intimate mixture of starch and finely powdered phosphorus pentachloride react, under anhydrous conditions at atmospheric pressure, to give chlorine derivatives of starch (60 to 63% Cl) (7). About ten equivalents of phosphorus pentachloride is used per glucose unit at an initial temperature of 100 to 105°C. (7) After 15 hours the temperature is increased to 125 to 135°C. and finally raised to 160 to 170°C. The suggested equation is (7):

$$C_6H_7O_2(OH)_3 + 5\ PCl_5 \rightarrow C_6H_5O_2Cl_5 + 5\ HCl + 3\ POCl_3 + 2\ PCl_3.$$

The proposed structure of the substituted glucose unit, accordingly, is believed to be that shown below (7).



A typical procedure is to grind together, under anhydrous conditions, 40 g. of dry starch (0.247 equivalent mole of anhydro-glucose) and 540 g. (2.59 moles) of phosphorus pentachloride (7). The mixture is then heated, at atmospheric pressure under anhydrous conditions, at 100 to 105°C. for 15 to 17 hours; then at 125 to 135°C. for 24 or more hours; and, finally, at 160 to 170°C. for eight hours or more (7). The product is then thoroughly washed with ice water, filtered, and dried (60 to 63% Cl) (7). This derivative is relatively stable and is believed to be a 1, 2, 3, 5, 6-*pentachlorostarch* (7).

8. Oxidized Derivatives of Starch. The oxidation of starch has been considered from two viewpoints; (*a*) the effect of various oxidizing agents under a variety of experimental conditions on the intact molecule as well as the mechanism of the oxidation, and (*b*) slight modifications, at a minimum cost, of the intact molecule in order to modify the physical properties and thus permit of new uses for these products. The first may be termed theoretical advances and the latter industrial advances. The bulk of work of the former type, particularly until recently, has been done in educational institutions, whereas the most of the work of the latter type has been done in industrial laboratories and is available only in part in the scientific literature. Oxidation of starch will be discussed in further detail in Chapter XI.

a. Theoretical Advances. During 1875 the oxidation of starch by reagents such as bromine (followed by silver oxide, chromic acid, potassium permanganate, and nitric acid) was studied by Lieben and Reichardt (142). A patent in 1892 claims that when starch is dissolved in an electrolyte such as a solution of hydrochloric acid, magnesium chloride, or sodium chloride and the mixture subjected to the passage of an electric current, a less viscous solution or complete solubility of the starch results (105). One year later (1893) the chemical and physical properties of six starches were reported (216).

The effect of bleaching powder, chloric acid, chromic acid, manganese dioxide, and potassium dioxide on starch was studied in 1896 (217). It was observed that starch treated with potassium permanganate forms a paste which is more liquid and transparent than is that prepared from ordinary starch (217). Chlorates, in conjunction with an acid in the presence of a catalyst, such as copper sulfate or vanadium chloride, were observed in 1901 to effect the solubility of starch (4). In the following year (1902) persulfates were used to solubilize starch (35).

During 1905 Wolff prepared a modified starch (which is immediately soluble in dilute alkaline solution at 70°C.) by the treatment of potato starch with acid oxidizing solutions at room temperature (263). Four years later (1909) the use of perborates to solubilize starch was reported (82). A paper in 1921 indicates that starch is not attacked by chlorine dioxide (ClO_2) (220). It was observed in 1924 that activin (sodium *p*-toluenesulfochloramide) effects the solubilization of starch without appreciable degradation to dextrins or sugars (74). Chloramines and oxidizing agents were used in 1927 on cold swelling starches (103). The oxidation of starch in the presence of ammonia and other bases yields a modified starch which is suitable for textile finishes (159). The oxidation of the hydrolytic products, glucose and maltose, has been studied under a variety of experimental conditions but does not form a part of this restricted topic (50, 51).

Reagents used in the oxidation of starch include (198): air (181), bleaching powder (61), bromine (58), chloramines (103), chloric acid (217), chlorates (4), chlorine (91), chlorine dioxide (220), chromic acid (93), ferric chloride (55), fluorine (165), halogen (2), hydrogen peroxide (3), hypochlorite (41), manganese dioxide (217), nitric acid (186), nitrogen dioxide (158), perborates (82), periodic acid (124), persulfates (35), potassium dioxide (92), potassium permanganate (139), silver oxide (92), *p*-toluenesulfochloramide, and zinc oxide (182).

b. Air as an Oxidizing Agent. Air was used, in the presence of metallic ions in alkaline solution, in 1925 to effect the oxidation of starch (181). The use of ferrous ion in alkali, for example, catalyzes the oxidation of starch by air to yield carbon dioxide and water as the principal products (181). Since the solution is alkaline, it is assumed that oxidation precedes any appreciable amount of hydrolysis of the starch molecule. Zinc oxide, in the presence of sunlight, also promotes the oxidation of starch (182). Inasmuch as ozone has no apparent action on starch, it may be used to bleach starch or flour (137).

c. Chromic Acid as an Oxidizing Agent. In 1893 it was observed that the oxidation of starch with chromic acid yields furfural (10%) (43). Such a drastic treatment is accompanied with, almost if not complete, hydrolysis of the starch molecule. The action of chromic acid on starch was studied again in 1896 (217).

Haerz observed (1905) that an *amylodextrin,* which he prepared by the oxidation of starch with chromic acid, consisted of a number of molecular groups of different complexity and density of internal structure (93). An *erythrodextrin* showed similar complexity, whereas an *achroodextrin* seemed to possess homogeneity (93). Chromic acid was used (1905) to solubilize starch (93).

In 1923 Reychler prepared a stable, soluble starch by the digestion of starch with a dilute solution of hydrochloric acid and potassium dichromate, subsequent reduction of the dichromate with sulfur dioxide, filtration of the residue, washing, and drying (203). When starch is treated with 0.25 *N* hydrochloric acid which contains 0.75% of potassium dichromate (or an equivalent weight of potassium permanganate) for twenty hours, washed, and dissolved in very dilute alkali, it gives a clear solution whereas water alone effects only swelling of the product (202, 203).

d. Halogen as an Oxidizing Agent. The first recorded observation relative to the oxidation of starch is by Liebig in 1829 that starch is slightly affected by the prolonged action of chlorine or chlorous acid (143). In 1874 Haberman reported the isolation of gluconic acid (and dextronic acid) from the products obtained by the oxidation of starch with reagents such as bromine or silver oxide (92). Seventeen years later (1891) Moissan reported that fluorine attacks starch with the deposition of carbon (165).

Four years later it was noted that a starch suspension, after an hour treatment with bromine in sodium hydroxide, reduces Fehling solution and gives a slight precipitate with phenylhydrazine, whereas neither bromine nor alkali alone produces such an affect (36). Chlorine gas was used in 1902 (105, 133), and a solution of chlorine and an alkali borate in 1926 to solubilize starch (91). The use of various halogen compounds to solubilize starch is disclosed in the patent literature (1). A 0.43% solution of hypochlorous acid effects an 80 to 90% solution of potato starch (41).

1. Oxidation of Rx·CHO to Rx·CO·OH, by Halogen. It is known that, in general, aldehydes are oxidized more readily than are alcohols, and primary alcohols are oxidized (as a consequence of two available hydrogen atoms) more readily than are secondary alcohols. In the oxidation of the monosaccharides, there is considerable evidence indicative that the lactol ring remains intact in the normal oxidation of an aldohexose to the corresponding aldonic acid (121). This suggests that the groups most susceptible to oxidation in the starch molecule are the terminal or free aldehyde groups.

It is generally believed, accordingly, that the acid groups produced during the mild oxidation of starch result from the oxidation of the terminal aldehyde groups to the corresponding carboxyl groups (210). The oxidation of the aldohexoses, by use of alkaline hypohalites, to the corresponding aldonic acids is the basis of an analytical procedure (262).

Alkaline hypochlorites are used in the commercial production of certain modified starches (224). The nature and extent of oxidation is not known. It seems relatively certain that some of the initial oxidation products are not uronic acids (67). Hypoiodite in methanol, for example, is used to oxidize the aldomonosaccharides to the corresponding aldonic acid for subsequent characterization as derivatives of benzimidazole (166). This reaction has been used also to determine the relative chain length of dextrins, which are produced by the action of α-amylase (171) and of hydrochloric acid on corn starch (141).

2. Oxidation of $Rx{\cdot}CH_2{\cdot}OH$ to $Rx{\cdot}CO{\cdot}OH$, by Halogen. An *amylodextrinic acid* was obtained during 1925 by the oxidation of *amylodextrin* with bromine in the presence of a mild base such as barium carbonate (240). The product gave a positive naphthoresorcinol (243) test and yielded furfural when distilled with hydrochloric acid (240). This derivative is considered to be a polyanhydroglucuronic acid.

When bromine is used as the oxidizing agent, as much as half of the oxidized starch may be polyanhydroglucuronic acids (67). The cinchonine salt of glucuronic acid, obtained by the acid hydrolysis of an oxidized starch, has been isolated and characterized (65). These products appear to be somewhat comparable to those obtained by the oxidation of starch in an organic solvent by the use of nitrogen dioxide (158).

3. Oxidation of $Rx{\cdot}CHOH{\cdot}Rz$ to $Rx{\cdot}CO{\cdot}Rz$, by Halogen. In certain oxidation reactions of the aldohexoses, it is believed that α-ketoaldonic acids are formed as intermediates (115). Such intermediates are probably formed in the air oxidation of the simple sugars in the presence of an alkaline solution of iron pyrophosphate (51). It is to be expected, then, that under certain oxidative conditions, some of the secondary hydroxyl groups in the starch molecule will be oxidized to ketone groups.

Bromine in acid solution has been studied as an oxidizing agent on about fifty carbohydrates (58). In the case of starch, the data indicate some oxidation of the secondary hydroxyl groups to ketone groups (58). This type of oxidation is indicated also by the formation of oximes by certain oxidized starches (65) and by the reducing power of some oxidized starches, at certain stages of the oxidation, against Fehling solution (67). The nitrogen analysis on the oxime of a certain oxidized starch is equivalent to about three ketone groups for every four anhydroglucose units (65).

4. *Oxidation of α, β-Secondary Alcohol Groups to Carbonyl or Carboxyl Groups, by Halogen.* Under proper experimental conditions, particularly in the use of periodic acid, the α, β-hydroxyl groups in a polyanhydroglucopyranose structure are oxidized to ketone groups with subsequent cleavage and oxidation to yield carboxyl groups. A decrease in optical rotation and an increase in the calcium content of the acid salts obtained, when starch is oxidized with bromine, indicate this particular type of oxidation and cleavage. Upon further oxidation and hydrolysis of these products, salts of diacids are obtained, which contain less than six carbon atoms (65). This general type reaction is believed to take place also in the production of the hexachlorostarches (244).

e. Nitric Acid and Nitrogen Dioxide as Oxidizing Agents. In 1838 Pelouze showed that oxalic acid is one of the products obtained by the oxidation of starch with concentrated nitric acid (186). During 1892 Petit subjected starch to prolonged treatment with nitric acid at 40°C. (189). Addition of water to the mixture yielded carbon dioxide, oxides of nitrogen, and a reducing acid ($C_5H_6O_5$) (189). This acid, on prolonged boiling, yielded a new acid ($C_5H_8O_6$) (189). These products suggest that hydrolysis precedes or accompanies oxidation. The reaction may be, essentially, one for the oxidation of monosaccharides.

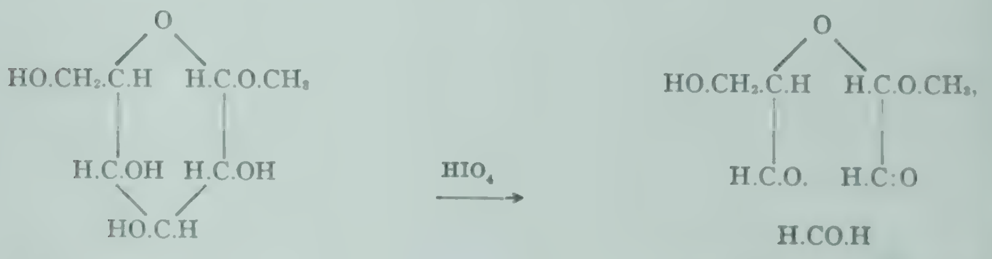
Mench and Degering, in 1942, oxidized dry starch, suspended in an inert organic solvent, with nitrogen dioxide to the polyglucuronic acid (158). By this laboratory procedure 16.5 g. (0.1 mole) of dry starch is added to 150 ml. of carbon tetrachloride in a 250 ml. three-neck flask, which is fitted with an efficient mechanical stirrer, a reflux condenser, and a water-jacketed burette (158). The nitrogen dioxide, in calculated amount from density and temperature data, is added dropwise through the burette, accompanied by efficient agitation of the mixture (158). The temperature is maintained at 25 to 29°C. until oxidation is complete. The product is then filtered on a pyrex fritted-glass funnel, washed well with carbon tetrachloride, freed of solvent on a vacuum filter, washed well with distilled water until free of traces of acid, washed with acetone, and then washed with ether. The product is then dried in a dessicator over phosphorus pentoxide (yield, 16 g. or more).

The carboxyl content is determined by titration with standard alkali (253), and the polyglucuronic acid is identified qualitatively by use of the β-naphthol test (243).

These oxidized starches, even those with relatively low carboxyl content, are insoluble in water but dissolve readily in warm, dilute bases such as dilute ammonium hydroxide, aqueous pyridine, aqueous Triton B, aqueous sodium carbonate (2%), aqueous sodium hydroxide (2%), and similar bases. They dissolve in a 5% solution of sodium bicarbonate with the liberation of carbon dioxide. They show no sign of retrogradation when relatively concentrated solutions of their salts are cooled. When these solutions are poured into alcohol, precipitation of the salts result. Salts of the heavy metals are obtained by treatment of the oxidized starches with aqueous solutions of the metal acetates.

The salts of these polyanhydroglucuronic acids may replace certain other modified starches as dispersants, gel formers, stabilizers, thickening agents, and in similar uses. The free acids may be used as acidulants, as disintegrates in certain food and medicinal products, as dispersants, and as a source of glucuronic acid.

f. Periodic Acid as an Oxidizing Agent. The first use of periodic acid as an oxidizing agent in carbohydrate chemistry is that by Malaprade in 1928 in a study of the ring structures of various methyl glycosides (156). These studies led to the conclusion that the alkyl aldohexopyranoside molecule is oxidized to a dialdehyde with the formation of one mole of formaldehyde per aldose unit, in accordance with the equation (104):



Methyl α-D⁺-glucoside **Dialdehyde + Formaldehyde**

This postulate was confirmed in 1936 when methyl α-D-mannoside was oxidized with aqueous barium hypobromite to a diacid, which was isolated and characterized as its strontium salt ($C_6H_8O_7Sr$) (122). Three other methyl D-glycosides gave the same derivative (122). This salt is obtained also by the oxidation of the glycoside with periodic acid to the 2, 4-dialdehyde and subsequent oxidation with bromine in aqueous strontium carbonate (122).

The treatment of ungelatinized corn starch with an aqueous solution of periodic acid (0.58 *M*) at room temperature for 24 hours, results in the reduction of about one molecular equivalent of periodic acid per glucose unit (123). This is the theoretical amount required to effect oxidation of the starch to the dialdehyde stage. Continued treatment with the oxidant results in a greatly diminished rate of oxidation, which indicates that the initial stage of the oxidation is complete at the dialdehyde stage (123).

Subsequent studies have indicated that the terminal glucose unit is further oxidized to give two moles of formic acid and one mole of formaldehyde whereas the terminal glycosyl unit yields one mole of formaldehyde (124). Subsequent hydrolysis, then, should yield two parts of formaldehyde, two parts of formic acid, one part of glyceraldehyde, one part of malonaldehyde, $n + 1$ parts of glyoxal, and n parts of L^+ – erythrose.

g. Permanganate as an Oxidizing Agent. In 1868 Langbein showed that 2 g. of starch will effect the reduction of 3 g. of potassium permanganate, but made no attempt to identify the end products of the reaction (139). The treatment (1890) of starch with potassium permanganate, in varying concentrations, results in the evolution of carbon dioxide, the precipitation of manganese dioxide, and the formation of a series of gummy acids, which may be precipitated by barium hydroxide or by lead acetate (149).

A soluble starch is prepared by the treatment of starch with a solution of potassium permanganate, then with dilute hydrochloric acid, and finally with sulfur dioxide or dilute bisulfite solution to remove the excess chlorine (217, 225).

Urea was obtained (1921) in a 20% yield by heating starch in an aqueous solution of ammonical copper oxide and calcium permanganate (70). The oxidation of starch (1922) with calcium permanganate, in the presence of ammonium hydroxide and mercuric oxide or silver nitrate, yields hydrogen cyanide (71). A clear, semi-fluid, transparent paste is obtained by the treatment of starch with dilute permanganate solution and subsequent decoloration with sulfur dioxide (226).

h. Peroxide as an Oxidizing Agent. Wurster observed, in 1889, that starch is not affected by hydrogen peroxide at room temperature but that it is hydrolyzed into glucose and dextrin by hydrogen peroxide in boiling acid or alkaline solution (268).

Asboth reported in 1892 that the treatment of starch with hydrogen peroxide, in the presence of ammonia, results in liquefaction with the evolution of carbon dioxide and oxygen (3). The reaction mixture is filtered and the products in the filtrate precipitated fractionally by the use of alcohol and barium hydroxide (3). Five years later (1897) soluble starch was prepared by the treatment of starch with a cold solution of sodium peroxide (238, 239). An increase in rotation was observed with an increase in the concentration of the sodium peroxide (238, 239).

In 1898 the solution of starch was effected by the use of sodium peroxide (238, 239), and 11 years later (1909) the use of acids, alkalies, and salts were suggested as catalysts in the oxidation of starch with hydrogen peroxide (68). It was observed that hydrogen peroxide alone, in large excess, effects complete liquefaction. During 1910, *achroodextrin, erythrodextrin,* and maltose were obtained by the action of a dilute solution of hydrogen peroxide on starch at 37°C. (90).

The action of hydrogen peroxide on starch affects the activity of amylase (90). Strong, transparent sizes, which are insoluble in water after drying, are obtained by the proper treatment of starch with neutral hydrogen peroxide (11, 90). In 1913 starch was solubilized by hydrogen peroxide in the presence of ferric chloride, but when these reagents are used individually no appreciable change occurs (52, 55).

A mixture of ferric chloride and hydrogen peroxide, was used in 1913, to effect an appreciable degradation of starch, whereas the affect of either used singly is much less (55). Years later (1919) it was reported in a paper that 60% aqueous hydrogen peroxide solution dissolves starch to give dough-like products or viscous solutions (6). In 1936 the hydrogen peroxide oxidation of starch, in the presence of ferrous ion, was studied by Brown, who concluded that hydrolysis precedes oxidation and that the reaction does not represent a true oxidation of the starch molecule (28).

BIBLIOGRAPHY

1. Anchors, G. R., U. S. Patent 1,329,353 (1920).
2. Anon., *T.I.B.A.*, **13**, 633 (1935).
3. Asboth, A. von, *Chem. Ztg.*, **16**, 1517, 1560 (1892).
4. Ashworth, A., British Patent 19,720 (1901).
5. Ballot, B., *Rap. ann. prog. chim.*, **4**, 222 (1844).
6. Bamberger, M., and Nussbaum, J., *Monatsh.*, **40**, 417 (1920).
7. Barham, H. N., Stickley, E. S., and Caldwell, M. J., Kansas State College, Unpublished (1945).
8. Barnett, W. L., *J. Soc. Chem. Ind. London,* **40**, 8T (1921).
9. Bayer, F., German Patent 363,192 (1920); 368, 413 (1920).
10. Béchamp, A., *Compt. rend.*, **37**, 134 (1853); **51**, 255 (1860); *Ann. chim. phys.*, [3] **64**, 311 (1862).
11. Bergh, F. P., and Neuberger, H. C., U. S. Patent 1,133,914 (1915); Sunder, C., *Bull. soc. ind. Mulhouse,* **90**, 558 (1924).
12. Bergmann, M., and Beck, F., *Ber.*, **54B**, 1574 (1921).
13. Bergmann, M., and Knehe, E., *Ann.*, **452**, 141 (1927).
14. Bergmann, M., and Ludewig, S., *Ber.*, **57B**, 961 (1924).
15. Berl, E., and Bütler, R., *Z. ges. Schiess-u. Sprengstoffw.*, **5**, 82 (1910).
16. Berl, E., and Bütler, R., *J. Soc. Chem. Ind. London*, **29**, 373 (1910).
17. Berl, E., and Kunze, W. C., *Ann.*, **520**, 270 (1935).
18. Berl, E., and Smith, W., *J. Soc. Chem. Ind. London*, **27**, 534 (1908).

19. Berthelot, *Ann. chim. phys.,* **9**, 316 (1876); *Compt. rend.,* **100**, 314 (1885).
20. Berthon, P., U. S. Patent 1,651,366 (1927).
21. Blondeau, C., *Rev. sci. ind. Paris,* **15**, 69 (1843).
22. Boeseken, J., Berg, J., C., and Kerstjens, A. H., *Rec. trav. chim.,* **35**, 320, 337 (1915).
23. Boettinger, C., *Arch. Pharm.,* **233**, 287 (1895).
24. Braconnot, H., *Ann. chim. phys.,* [2] **52**, 290 (1833).
25. Brigl, P., and Schinle, R., *Ber.,* **62B**, 99 (1929).
26. Brimhall, B., and Hixon, R. M., *Cereal Chem.,* **19**, 425 (1942).
27. Brown, H. T., and Millar, J. H., *J. Chem. Soc.,* **75**, 308 (1899).
28. Brown, W. R., *J. Biol. Chem.,* **113**, 417 (1936).
29. Bruson, H. A., U. S. Patent 2,401,607 (1946).
30. Burkhard, C. A., and Degering, E. F., *Rayon Textile Monthly,* **23**, 340 (1942).
31. Burkhard, C. A., and Degering, E. F., *Rayon Textile Monthly,* **23**, 416 (1942).
32. Burkhard, C. A., and Degering, E. F., *Proc. Indiana Acad. Sci.,* **51**, 173 (1942).
33. Caesar, G. V., and Goldfrank, M., *J. Am. Chem. Soc.,* **68**, 372 (1946); Caesar, G. V., U. S. Patent 2,365,173 (1944); U. S. Patent 2,400,287 (1946).
34. Carolles, B., *Rev. sci. ind. Paris,* **15**, 69 (1843).
35. Cerf. L., U. S. Patent 698,632 (1902).
36. Chalmot, G. de, *Am. Chem. J.,* **17**, 535 (1895).
37. Chowdhury, J. K., *Biochem. Z.,* **148**, 76 (1924).
38. Clark, H. T., and Gillespie, H. B., *J. Am. Chem. Soc.,* **54**, 2083 (1932).
39. Coehn, A., *Z. Electrochem.,* **4**, 63 (1897); Bottazzi, F., and Victorow, C., *Atti accad. Lincei,* **19**, II, 7 (1910); *Chem. Abstracts,* **5**, 1406 (1911); Gruzewska, Z., *J. physiol. path. gén.,* **14**, 7 (1912); *Chem. Abstracts,* **7**, 89 (1913).
40. Cope, W. C., and Barab, J., *J. Am. Chem. Soc.,* **39**, 504 (1917).
41. Craik, J., *J. Soc. Chem. Ind.,* **43**, 171T (1924); Seck, W., British Patent 437,890 (1935).
42. Cramer, F. B., Gardner, T. S., and Purves, C. B., *Ind. Eng. Chem., Anal. Ed.,* **15**, 319 (1943).
43. Cross, C. F., Bevan, E. J., and Beadle, C., *Ber.,* **26**, 2520 (1893).
44. Cross, C. F., Bevan, E. J., and Beadle, C., *J. Chem. Soc.,* **63**, 837 (1893).
45. Cross, C. F., Bevan, E. J., and Briggs, J. F., *J. Chem. Soc.,* **91**, 612 (1907).
46. Cross, C. F., Bevan, E. J., and Traquair, J.. *Chem. Ztg.,* **29**, 527 (1905).
47. Damansky, A. F., *Compt. rend. soc. biol.,* **114**, 1051 (1933).
48. Damansky, A. F., *Ann. chim.* [11] **2**, 491 (1934).
49. Degering, E. F., An Outline of the Chemistry of the Carbohydrates, John F. Swift Co., Cincinnati, 73, 75 (1939).
50. Degering, E. F., *J. Biol. Chem.,* **95**, 409 (1932); *Proc. Indiana Acad. Sci.,* **43**, 105 (1934).

50a. Degering, E. F., and Rankin, D., *Proc. Indiana Acad. Sci.,* **54**, 114 (1945).

51. Degering, E. F., and Upson, F. W., *J. Biol. Chem.,* **94**, 423 (1931).
52. Dhar, N. R., *J. Phys. Chem.,* **28**, 943 (1924); Perkins, F. G., U. S. Patent 1,020,656 (1912).
53. Dreyfus, H., British Patent 166,767 (1920).
54. Du Pont, British Patent 454,963 (1936); U. S. Patent 2,111,867 (1934).
55. Durieux, O., *Bull. soc. chim. belg.,* **27**, 90 (1913).
56. Elssner, R., U. S. Patent 2,000,887 (1935).
57. Escales, R., and Levy, H., *Kunststoffe,* **13**, 25, 52, 64 (1923); *Chem. Abstracts,* **18**, 2248 (1924).

58. Everett, M. R., and Sheppard, F., Oxidation of Carbohydrates in Acid Solution, Univ. of Oklahoma Medical School, Oklahoma City, Oklahoma (1936).
59. Farbenindustrie, German Patent 484,242 (1923).
60. Farbenindustrie, German Patent 478,127 (1924).
61. Farbenindustrie, German Patent 538,839 (1924).
62. Farbenindustrie, German Patent 492,319 (1926); French Patent 640,174 (1927); British Patent 279,864 (1926); 293,316 (1927); 293,757 (1927); French Patent 656,861 (1928); British Patent 318,967 (1928); 326,865 (1928); 355,144 (1929); 356,665 (1929); U. S. Patent 1,928,269 (1933).
63. Farbenindustrie, British Patent 359,618 (1929); German Patent 550,428 (1929).
64. Farbenindustrie, British Patent 283,181 (1927); 297,766 (1927); U. S. Patent 1,940,589 (1930); French Patent 668,686 (1929).
65. Farley, F. F., and Hixon, R. M., *Ind. Eng. Chem.*, **34**, 677 (1942).
66. Fehling, H., *Ann.*, **55**, 13 (1845).
67. Felton, G., Farley, F. F., and Hixon, R. M., *Cereal Chem.*, **15**, 678 (1938).
68. Fernbach, A., and Wolff, J., *Proc. Intern. Cong. Appl. Chem.*, **6b**, 124 (1909).
69. Fordyce, C. R., and Meyer, L. W. A., *Ind. Eng. Chem.*, **32**, 1053 (1940).
70. Fosse, R., *Bull. soc. chim.*, [4] **29**, 158 (1921).
71. Fosse, R., *Compt. rend. soc. biol.*, **86**, 175 (1922).
72. Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.*, **66**, 557 (1944).
73. Franchimont, A., *Compt. rend.*, **89**, 711 (1879).
74. Frere, J., *Revue prod. chim.*, **27**, 721 (1924); Haller, R., *Melliand Textileber.*, **5**, 389 (1924).
75. Freudenberg, K., *Naturwissenschaften*, **17**, 959 (1929); Staudinger, H., Kern, W., and Herrera, J. J., *Ber.*, **68B**, 2346 (1935).
76. Freudenberg, K., Boppel, H., and Meyer-Delius, M., *Naturwissenschaften*, **26**, 123 (1938).
77. Freudenberg, K., and Harder, M., *Ann.*, **433**, 230 (1923).
78. Freudenberg, K., and Rapp, W., *Ber.*, **69B**, 2041 (1936).
79. Freudenberg, K., Schaaf, E., Dumpert, G., and Ploetz, T., *Naturwissenschaften*, **27**, 850 (1939).
80. Friedel, C., *Ber.*, **2**, 163 (1869).
81. Friese, M., *et al.*, *Ber.*, **61B**, 1975 (1928).
82. Fritsche, F., U. S. Patent 910,524 (1909); Stolle, and Kopke, French Patent 384,704 (1907); Austrian Patents 37,835, 37,836, 40,449; German Patent 202,229 (1907).
83. Fukushima, I., and Takamatsu, Y., *J. Soc. Chem. Ind. Japan*, **32**, 130 (1929); *Chem. Abstracts*, **23**, 5058 (1929).
84. Gault, H., *Compt. rend.*, **177**, 592 (1923).
85. Gault, H., and Ehrmann, M. P., *Chimie & industrie*, *Special No.*, 574 (May, 1924).
86. Genin, G., *Rev. gén. mat. plastiques*, **12**, 113 (1936).
87. Genung, L. B., and Mallatt, R. C., *Ind. Eng. Chem., Anal. Ed.*, **13**, 369 (1941).
88. Gomberg, M., and Buchler, C. C., *J. Am. Chem. Soc.*, **43**, 1904 (1921).
89. Gottlieb, D., Caldwell, C. G., and Hixon, R. M., *J. Am. Chem. Soc.*, **62**, 3342 (1940).
90. Gruzewska, Z., *Compt. rend. soc. biol.*, **68**, 1084 (1910); Gerber, C., *Compt. rend. soc. biol.*, **72**, 1002 (1912); *Compt. rend.*, **154**, 1543 (1912).
91. Haake, W., German Patent 547,421 (1926); Höppler, F., German Patent 558,145 (1929).
92. Haberman, J., *Ann.*, **172**, 11 (1874)

93. Haerz, E., *Wochschr. Brau.,* **22**, 721 (1905).
94. Hagedorn, M., U. S. Patent 1,994,608 (1935).
95. Halen, S., *Kunststoffe,* **11**, 121 (1921).
96. Hamilton, R. M., and Yanovsky, E., *Ind. Eng. Chem.,* **38**, 864 (1946).
97. Hassid, W. Z., and Dore, W. H., *J. Am. Chem. Soc.,* **59**, 1503 (1937).
98. Hawkins, E. G. E., Jones, J. K. N., and Young, G. T., *J. Chem. Soc.,* 390 (**1940**).
99. Haworth, W. N., Hirst, E. L., and (a) Kitchen, H., and Peat, S., *J. Chem. Soc.,* 791 (**1937**); (b) Plant, M. M. T., *J. Chem. Soc.,* 1214 (**1935**); (c) Waine, A. C., *J. Chem. Soc.,* 1299 (**1935**); also see Hirst, E. L., Plant, M. M. T., and Wilkinson, M. D., *J. Chem. Soc.,* 2375 (**1932**).
100. Haworth, W. N., Hirst, E. L., and Webb, J. I., *J. Chem. Soc.,* 2681 (**1928**); Baird, D. K., Haworth, W. N., and Hirst, E. L., *J. Chem. Soc.,* 1201 (**1935**).
101. Haworth, W. N., Hirst, E. L., and Woolgar, M. D., *J. Chem. Soc.,* 177 (**1935**).
102. Helferich, B., and Koester, H., *Ber.,* **57B**, 587 (1924).
103. Henkel et cie, Ges., British Patent 289,053 (1927).
104. Herissey, H., Fleury, P., nd Joly, M., *J. pharm. chim.,* **20**, 149 (1934).
105. Hermite, F., British Patent 1,061 (1892); German Patent 70,275 (1892); Siemens, and Halske, German Patent 88,447 (1895); U. S. Patent 798,509 (1905).
106. Hess, K., and (a) Eveking, W., *Ber.,* **67B**, 1908 (1934); (b) Littmann, O., *Ber.,* **67B**, 465 (1934); (c) Littmann, O., and Pfleger, R., *Ann.,* **507**, 55 (1933).
107. Hess, K., and Pfleger, R., *Ann.,* **507**, 48 (1933).
108. Hess, K., Pfleger, R., and Trogus, C., *Ber.,* **66B**, 1505 (1933).
109. Hess, K., and Smith, F. A., *Ber.,* **62B**, 1619 (1929).
110. Higginbotham, R. S., and Richardson, W. A., *J. Soc. Chem. Ind. London,* **57**, 234 (1938).
111. Hirst, E. L., and Young, G. T., *J. Chem. Soc.,* 1471 (**1939**).
112. Hoenig, M., et al., *Monatsh.,* **7**, 455 (1886).
113. Hoenig, M., and Schubert, S., *Monatsh.,* **6**, 708 (1885). See also ref. 114.
114. Hoenig, M., and Schubert, S., *Monatsh.,* **7**, 455 (1886).
115. Hoenig, M., and Tempus, F., *Ber.,* **57B**, 787 (1924); *Chem. Abstracts,* **18**, 2872 (1924).
116. Holberger, J. H., German Patent 662,180 (1932).
117. Houghton, A. A., British Patent 493,513 (1938).
118. Hughes, G. K., Macbeth, A. K., and Winzor, F. L., *J. Chem. Soc.,* 2026 (**1932**).
119. Hurd, C. D., Cantor, S. M., and Roe, A. S., *J. Am. Chem. Soc.,* **61**, 426 (1939).
120. Irvine, J. C., and MacDonald, J. *J. Chem. Soc.,* 1502 (**1926**).
121. Isbell, H. S., *J. Research Natl. Bur. Standards,* **8**, 327 (1932); Isbell, H. S., and Frush, H. L., *Chem. Abstracts,* **29**, 4679 (1935).
122. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **58**, 378 (1936); **59**, 994 (1937).
123. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **59**, 2049 (1937).
124. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **60**, 989 (1938); Caldwell, C. G., and Hixon, R. M., *J. Biol. Chem.,* **123**, 595 (1938).
125. Kalinowsky, J. von, *J. prakt. Chem.,* **35**, 193 (1845).
126. Karrer, P., *Helv. Chim. Acta,* **3**, 620 (1920).
127. Karrer, P., and Fioroni, W., *Ber.,* **55B**, 2857 (1922).
128. Karrer, P., and von Krauss, E., *Helv. Chim. Acta,* **12**, 1151 (1929).
129. Karrer, P., and Zega, Z., *Helv. Chim. Acta,* **6**, 822 (1923).
130. Kerb, J., *Biochem. Z.,* **100**, 3 (1919).

131. Kerr, R. W., Trubell, O. R., and Severson, G. M., *Cereal Chem.*, **19**, 64 (1942); Kerr, R. W., and Trubell, O. R., *Cereal Chem.*, **18**, 530 (1941).
132. Kesseler, K., and Röhm, R., *Z. angew. Chem.*, **35**, 125 (1922).
133. Kindscher, H., German Patent 168,980 (1902); Watson, W., and Kent-Jones, D. W., U. S. Patent 1,519,014 (1924); MacMillan, J. R., U. S. Patent 1,567,609 (1925); Bergquist, C., British Patent 294,979 (1927); Intern. Pat. Devel. Co., French Patent 657,605 (1928); Bergquist, C., U. S. Patent 1,851,749 (1932); 1,871,027 (1932).
134. Kindt, *Ann. Physik.*, **70**, 167 (1847).
135. Kldiashwili, A. G., *J. Chem. Soc.*, **86**, Part 1, 798 (1904); **88**, Part 1, 634 (1905).
136. Kldiashwili, A. G., *J. Russ. Phys. Chem. Soc.*, **36**, 905 (1904); **37**, 421 (1905).
137. Koenig, L., *J. Chem. Soc. Ind.*, **13**, 824 (1894).
138. Kraemer, E. O., *Ind. Eng. Chem.*, **30**, 1200 (1938).
139. Langbein, G., *Z. Russland*, **7**, 573 (1868).
140. Law, D. J., *Chem. Ztg.*, **32**, 365 (1908).
141. Levine, M., Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.*, **64**, 2331 (1942).
142. Lieben, A., and Reichardt, *Ber.*, **8**, 1020 (1875).
143. Liebig, J., *Poggendorff's Ann. Phys. Chem.*, **15**, 541 (1829).
144. Liechti, L., and Suida, W., *Ber.*, **16**, 2453 (1883).
145. Lilienfeld, L., British Patent 181,393 (1922); German Patent 475,884 (1921); Austrian Patent 82,866 (1921).
146. Lilienfeld, L., German Patent 360,415 (1922); also see British Patent 12,854 (1912); 6,035 (1913); 6,387 (1913); 163,016 (1920); 163,017 (1920); 200,815 (1923); 231,800 (1924); 231,805 (1924); Canadian Patent 222,377 (1922); U. S. Patent 1,350,820 (1920).
147. Lilienfeld, L., French Patent 676,344 (1929); French Patent 699,217 (1930); Harrison, W., German Patent 497,240 (1927).
148. Link, K. P., Niemann, C. G., and Roberts, R. H., U. S. Patent 2,121,919 (1938).
149. Lintner, C. J., *Z. angew. Chem.*, **3**, 546 (1890).
150. Lolkema, J., Dutch Patent 55,779 (1944).
151. Lolkema, J., and Moller, F. A., Dutch Patent 56,340 (1944).
152. Lorand, E., *Cellulosechemie*, **13**, 185 (1932).
153. Lorand, E., U. S. Patent 1,959,590 (1934).
154. Mack, D. E., and Shreve, R. N., *Ind. Eng. Chem.*, **34**, 304 (1942).
155. Maksorov, B. V., and Andrianov, K. A., Russian Patent 39,098 (1934); *Rev. gén. mat. plastiques*, 11, 336, 373, 375 (1935); *Plasticheskie Massy*, **6**, 1 (1933).
156. Malaprade, L., *Bull. soc. chim.* **43**, 683 (1928); Karrer, P., and Pfaehler, K., *Helv. Chim. Acta*, **17**, 766 (1934).
157. McBurney, J. D., and Nollau, E. H., British Patent 278,696 (1926).
158. Mench, J. W., with Degering, E. F., *Proc. Indiana Acad. Sci.*, **55**, 69 (1946).
159. Meulen, J. H., van der, German Patent 624,988 (1936).
160. Meyer, K. H., Natural and Synthetic High Polymers, Interscience Publishers, New York, 398 (1942).
160a. Meyer, K. H., Bernfeld, P., and Hohenemser, W., *Helv. Chim. Acta*, **23**, 885 (1940).
161. Meyer, K. H., Hopff, H., and Mark, H., *Ber.*, **62B**, 1103 (1929); Freudenberg, K., Kuhn, W., Dürr, W., Bolz, F., and Steinbrunn, G., Ber., **63B**, 1510 (1930); Kuhn, W., *Ber.*, **63B**, 1503 (1930).
162. Meyer, K. H., and Wolff, E., *Kolloid-Z.*, **89**, 194 (1939); Staudinger, H., and Fischer, K., *J. prakt. Chem.*, **157**, 19 (1940); Meyer, K. H., *Kolloid-Z.*, **95**, 70 (1941); Schulz, G. V., and Dinglinger, A., *J. prakt. Chem.*, **158**, 136 (1941).

163. Michael, A., *Am. Chem. J.,* **5**, 359 (1883).
164. Middleton, E. B., U. S. Patent 1,685,220 (1928).
165. Moissan, H., *Ann. chim. phys.,* [6] **24**, 224 (1891).
166. Moore, S., and Link, K. P., *J. Biol. Chem.,* **133**, 293 (1940).
167. Mühlhauser, O., *Dinglers Polytech. J,* **284**, 137 (1892).
168. Mullen, J. W., and Pacsu, E., *Ind. Eng. Chem.,* **34**, 807 (1942).
169. Mullen, J. W., and Pacsu, E., *Ind. Eng. Chem.,* **34**, 1209 (1942).
170. Mullen, J. W., and Pacsu, E., *Ind. Eng. Chem.,* **35**, 381 (1943).
171. Newton, J. M., Ph.D. Thesis, Iowa State College, Ames, Iowa (1941).
172. Nichols, P. L. Jr., and Hamilton, R. M., U. S. Patent 2,405,973 (1946).
173. Nichols, P. L. Jr., Hamilton, R. M., Smith, L. T., and Yanovsky, E., *Ind. Eng. Chem.,* **37**, 201 (1945).
174. Nichols, P. L. Jr., Meiss, P. E., and Yanovsky, E., U. S. Patent 2,413,463 (1946).
175. Nobel, A., German Patent 57,711 (1891).
176. Okada, H., *Cellulose Ind.,* **3**, 3 (1927).
177. Ost, H., Westhoff, F., and Gessner, L., *Ann.,* **382**, 340 (1911).
178. Pacsu, E., and Mullen, J. W., *J. Am. Chem. Soc.,* **63**, 1168 (1941); Degering, E. F., An Outline of the Chemistry of the Carbohydrates, John F. Swift Co., Cincinnati, pp. 109-258 (1943).
179. Pacsu, E., and Mullen, J. W., *J. Am. Chem. Soc.,* **63**, 1487 (1941).
180. Pacsu, E., and Mullen, J. W., U. S. Patent 2,372,337 (1945).
181. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.,* **29**, 799 (1925); **30**, 939 (1926); **34**, 711 (1930).
182. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.,* **32**, 1263 (1928).
183. Pancirolli, F., *Boll. reparto fibre tessili vegetali regia staz. sper. ind. carta e fibre tessili vegetali,* **32**, 314 (1937).
184. Payen, A., *Compt. rend.* **24**, 85 (1847).
185. Peiser, E., *Z. physiol. Chem.,* **161**, 210 (1926); **167**, 88 (1927).
186. Pelouze, J., *Compt. rend.,* **7**, 713 (1838).
187. Pelouze, J., *Compt. rend.,* **23**, 809, 892 (1846).
188. Penn, W. S., *Paint Manuf.,* **16**, 161 (1946).
189. Petit, P., *Compt. rend.,* **114**, 1375 (1892).
190. Pigman, W. W., and Wolfrom, M. L., *Advances in Carbohydrate Chem.,* **1**, 290, 292, 299 (1945).
191. Posternak, T., *Helv. Chim. Acta,* **18**, 1351 (1935).
192. Pregl, F., *Monatsh.,* **22**, 1049 (1901). cf. *J. Chem. Soc.,* 1201 (**1935**).
193. Pregl, F., *J. Soc. Chem. Ind.,* **21**, 129 (1902).
194. Pringsheim, H., and Eissler, F., *Ber.,* **46**, 2959 (1913).
195. Pringsheim, H., and Lassman, M., *Ber.,* **55B**, 1409 (1922).
196. Pringsheim, H., Leibowitz, J., and Silmann, S. H., *Ber.,* **58B**, 1889 (1925); Urbanski T., and Hackel, J., *Chem. Zentr.,* **I**, 1938 (1940); Snelling, W. O., and Rees, G. E., U. S. Patent 2,271,877 (1942).
197. Pringsheim, H., and Meyersohn, P., *Z. physiol. Chem.,* **173**, 211 (1928).
198. Radley, J. A., *Mfg. Chemist,* **13**, 101, 158, 201 (1942).
199. Reich, W. S., and Damansky, A. F., *Compt. rend.,* **196**, 1610 (1933); **197**, 275 (1933); *Compt. rend. soc. biol.,* **113**, 23 (1933).
200. Reich, W. S., and Damansky, A. F., *Bull. soc. chim. biol.,* **19**, 158, 357 (1937).
201. Reinsch, H., *Rep. Pharm.,* **3**, 6 (1849).
202. Reychler, A., German Patent 360,128 (1921).

203. Reychler, A., *Bull. soc. chim. belg.*, **32**, 221 (1923).
204. Ritter, F., *Dinglers Polytech. J.*, **161**, 146 (1861).
205. Rosenthal, L., and Lenhard, W., U. S. Patent 1,739,863 (1929).
206. Sadtler, S. S., *Chem. & Met. Eng.*, **16**, 361 (1917).
207. Sadtler, S. S., U. S. Patent 1,211,761 (1917); Helle, J., and Kunz, A., U. S. Patent 2,127,360 (1938).
207a. Sakurada, I., and Inoue, R., *J. Soc. Chem. Ind.*, **39**, suppl. binding, **48** (1936); *Chem. Abstracts*, **30**, 4714 (1936).
208. Samec, M., *Kolloidchem. Beihefte*, **6**, 23 (1914); Sherman, H. C., and Baker, J. C., *J. Am. Chem. Soc.*, **38**, 1885 (1916); Samec, M., and Haerdtl, H., *Kolloidchem. Beihefte*, **12**, 281 (1920); Samec, M., and Mayer, A., *Compt. rend.*, **172**, 1079 (1921); **173**, 321 (1921); *Kolloidchem. Beihefte*, **13**, 272 (1921); **16**, 89, 91 (1922).
209. Samec, M., Kolloidchemie der Stärke, Steinkopff, Leipzig (1927).
210. Samec, M., *Kolloid-Beihefte*, **28**, 155 (1929); Samec, M., *Kolloid-Z.*, **59**, 266 (1932); **64**, 321 (1933); Rassow, B., and Lobenstein, M., *Kolloid-Beihefte*, **33**, 179 (1931); Samec, M., and Blinc, M., *Kolloid-Beihefte*, **38**, 48 (1933).
211. Samec, M., *Kolloid-Beihefte*, **37**, 91 (1932).
212. Samec, M., (a) *Kolloid-Z.*, **59**, 266 (1932); **64**, 321 (1933); and (b) Knop, L., *Kolloid-Beihefte*, **39**, 438 (1934).
213. Samec, M., and Hoefft, F. von, *Kolloidchem. Beihefte*, **5**, 141 (1913).
214. Samec, M., and Mayer, A., *Kolloidchem. Beihefte*, **12**, 1228 (1920).
215. Sapozhnikov, A. V., *J. Russ. Phys.-Chem. Soc.*, **35**, 126 (1903).
216. Schaeffer, G., and Scheurer, O., *Bull. soc. ind. Mulhouse*, **63**, 363 (1893).
217. Schmerber, O., *Bull. soc. ind. Mulhouse*, **66**, 238 (1896); Dollfus, E., and Scheurer, F., *Bull. soc. ind. Mulhouse*, **66**, 241 (1896).
218. Schmid, L., *Ber.*, **58B**, 1963 (1925).
219. Schmid, L., and Zentner, M., *Monatsh.*, **49**, 111 (1928).
220. Schmidt, E., and Graumann, E., *Ber.*, **54**, 1860 (1921).
221. Schoch, T. J., *Cereal Chem.*, **18**, 121 (1941).
222. Schoch, T. J., *J. Am. Chem. Soc.*, **64**, 2954 (1942); Kerr, R. W., *Cereal Chem.*, **20**, 299 (1943); Whistler, R. L., and Hilbert, G. E., *J. Am. Chem. Soc.*, **66**, 1721 (1944).
223. Schützenberger, P., *Compt. rend.*, **61**, 485 (1865); **68**, 814 (1869); *Ann. chim. phys.*, [4] **21**, 235 (1870).
224. Shane, R. S., *J. Chem. Education*, **14**, 460 (1937).
225. Siemens, W. von, and Witt, C., British Patent 24,455 (1895); Bredt, O., British Patent 22,370 (1903); German Patent 14,958.
226. Sjostrom, O. A., Canadian Patent 356,463 (1936).
227. Skraup, Z. H., *Monatsh.*, **19**, 458 (1898); **26**, 1415 (1905); *Ber.*, **32**, 2413 (1899).
228. Snelling, W. O., and Boyd, R. N., U. S. Patent 1,873,061 (1932).
229. Staudinger, H., (a) *Ber.*, **59B**, 3031 (1926); and (b) Heuer, W., *Ber.*, **63B**, 222 (1930); (c) Schulz, G. V., *Ber.*, **68B**, 2320 (1935).
230. Staudinger, H., and Daumiller, G., *Ann.*, **529**, 219, 259 (1937).
231. Staudinger, H., and Eilers, H., *Ber.*, **69B**, 819 (1936).
232. Staudinger, H., and Husemann, E., *Ann.*, **527**, 195, 207 (1937).
233. Staudinger, H., and Husemann, E., *Ber.*, **71B**, 1057 (1938); Hirst, E. L., and Young, G. T., *J. Chem. Soc.*, 951 (**1939**).
234. Staudinger, H., and Schweitzer, O., *Ber.*, **63B**, 2323 (1930).

235. Stein, H., German Patent 573,191 (1931).
236. Stern, E., U. S. Patent 1,412,020 (1922).
237. Sutra, R., *Compt. rend.*, **195**, 1079 (1932); **196**, 1608 (1933); **198**, 1863 (1934).
238. Syniewski, W., *Ber.*, **30**, 2415 (1897); **31**, 1793 (1898).
239. Syniewski, W., *Ber.*, **31**, 1793 (1898); *Ann.*, **324**, 239 (1902).
240. Syniewski, W., *Ann.*, **441**, 277 (1925).
241. Tamba, R., *Biochem. Z*, **141**, 274 (1923).
242. Taylor, T. C., and Walton, R. P., *J. Am. Chem. Soc.*, **51**, 3437 (1929).
243. Thomas, M. P., *Bull. soc. chim. biol.*, **7**, 102 (1925).
244. Thomson, T. R., The Direct Chlorination of Starch, Thesis, Kansas State College (1945); Beutel, E., and Kutzelnigg, A., *Monatsh.*, **56**, 205 (1935); **66**, 248 (1935); *Chem. Abstracts*, **29**, 3825 (1935); **30**, 4665 (1936).
245. Timmermans, J., and Martin, F., *J. chim. phys.*, **23**, 733 (1926).
246. Tokuzo, N., Japanese Patent, 130,827 (1939); *Chem. Abstracts*, **35**, 2026 (1941).
247. Tomecko, C. G., and Adams, R., *J. Am. Chem. Soc.*, **45**, 2698 (1923).
248. Traquair, J., *J. Soc. Chem. Ind.*, **28**, 288 (1909).
249. Traube, W., Blaser, B., and Grunert, C., *Ber.*, **61**, 754 (1928); British Patents 294,572 and 322,003 (1928); French Patent 657,204 (1928).
250. Tsuzuki, Y., *Bull. Chem. Soc. Japan*, **3**, 276 (1928); **4**, 21, 153 (1929).
251. Tsuzuki, Y., *Chem. Abstracts*, **23**, 1393 (1929); **24**, 260 (1930).
252. Tsuzuki, Y., *Chem. Absracts*, **23**, 2425 (1929).
253. Unruh, C. C., and Kenyon, W. O., *J. Am. Chem. Soc.*, **64**, 127 (1942).
254. Walton, R. P., A Comprehensive Survey of Starch Chemistry, The Chemical Catalog Co., Inc., New York (1928).
255. West, C. J., *Paper*, **27**, 16, 36 (1920).
256. Winfield, J. R., and Ritchie, G. G., British Patent 535,949 (1941).
257. Whistler, R. L., and Hilbert, G. E., *Ind. Eng. Chem.*, **36**, 796 (1944).
258. Whistler, R. L., and Jeanes, A., *Ind. Eng. Chem., Anal. Ed.*, **15**, 317 (1943).
259. Whistler, R. L., Jeanes, A., and Hilbert, G. E., Abstracts, Buffalo Meeting of the American Chemical Society, September (1942).
260. Whistler, R. L., and Schieltz, N. C., *J. Am. Chem. Soc.*, **65**, 1436 (1943).
261. Will, W., and Lenze, F., *Ber.*, **31**, 68 (1898).
262. Willstätter, R., and Schudel, G., *Ber.*, **51**, 780 (1918).
263. Wolff, J., *Compt. rend.*, **141**, 1046 (1905).
264. Wolffenstein, R., and Oeser, E., Chem. *Zentr.*, **2**, 366 (1925).
265. Worden, E. C., *Kunstoffe*, **3**, 61 (1913). See also refs. 266 and 267.
266. Worden, E. C., Technology of the Cellulose Esters, London (1921).
267. Worden, E. C., Technology of the Cellulose Ethers, Newark Printing Company, Newark, N. J. (1933).
268. Wurster, C., *Ber.*, **22**, 145R (1889).
269. Young, G., British Patent 184,825 (1921).
270. Ziese, W., *Z. physiol. Chem.*, **229**, 213 (1934); **235**, 235 (1935).
271. Zulkowski, K., *Ber.*, **23**, 3295 (1890).

Supplementary References

A. Esters:

a. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.*, **66**, 111 (1944).
b. Bear, R. S., *J. Am. Chem. Soc.*, **64**, 1388 (1942).
c. Bear, R. S., and Cori, C. F., *J. Biol. Chem.*, **140**, 111 (1941).

d. Bley, R. S., U. S. Patent 2,140,346 (1938).
e. British Celanese Limited, British Patent 487,020 (1938).
f. Caesar, G. V., U. S. Patent 2,365,173 (1944).
g. Dejarme, N., *Rev. gén. mat. plastiques,* **14**, 107 (1938).
h. Doree, C., The Methods of Cellulose Chemistry, Chapman & Hall, London, 295 (1933).
i. Groen, M. G., Dutch Patent 56.009 (1944); U. S. Patent 2,412,213 (1946).
j. Hagedorn, M., and Reyle, B., U. S. Patent 2,009,657 (1935).
k. Haworth, W. N., Gregory, H., and Wiggins, L. F., *J. Chem. Soc.,* 488 (**1946**).
l. Knoevenagel, E., *Ann.,* **402**, 118 (1914).
m. Rudy, H., *Cellulosechemie,* **13**, 49 (1932).
n. Schmidt, A., Balle, C., and Lange, H., U. S. 1,732,306 (1932); U. S. Patent 1,928,269 (1933).
o. Staudinger, H. and Dreher, E., *Ann.,* **517**, 73 (1935).
p. Talley, E. A., and Smith, L. T., *J. Org. Chem.,* **10**, 101 (1945).
q. Treadway, R. H., U. S. Patent 2,399,455 (1946).
r. Treadway, R. H., and Yanovsky, E., *J. Am. Chem. Soc.,* **67**, 1038 (1945).

B. Ethers:

a. Frank, G., and Mienes, K., German Patent 555,930 (1931); 575,349 (1933).
b. Nichols, P. L., Jr., Wrigley, A. N., and Yanovsky, E., *J. Am. Chem. Soc.,* **68**, 2020 (1946).
c. Rankin, D., with Degering, E. F., The Preparation and Properties of Certain Ether Derivatives of Starch, Ph.D. Thesis, Purdue University, June, 1942.
d. Rohm and Haas Co., *J. Textile Inst.,* **36**, A49 (1945); British Patent 546,585 (1943).

C. Halogen Derivatives:

a. Barham, H. N., Stickley, E. S., and Caldwell, M. J., *J. Am. Chem. Soc.* **68**, 1018 (1946).
b. Haller, R., *Helv. Chim. Acta,* **29**, 1156, 1688 (1946).

D. Nitro Derivatives:

a. Ashford, W. R., Cooke, L. M., and Hibbert, H., *Can. J. Research,* **24B**, 238 (1946).
b. Ashford, W. R., Evans, T. H., and Hibbert, H., *Can. J. Research,* **24B**, 246 (1946).
c. Ashford, W. R., and Hibbert, H., *Can. J. Research,* **25B**, 151 (1947).
d. Holmes, F. B., U. S. Patent 779,421; 779,422 (1905).

E. Oxidized Derivatives:

a. Baker, W., and Field, F. B., *J. Chem. Soc.,* 86 (**1932**).
b. Brender, C., British Patent 17,650 (1898).
c. Bryant, A. P., U. S. Patent 1,937,543 (1933).
d. Fuller, A. D., U. S. Patent 1,942,544 (1934); 2,014,798 (1935); 2,173,878 (193[illegible]
e. Grangaard, D. H., Gladding, E. K., and Purves, C. B., *Paper Trade J.,* **115**, No. 7, 41 (1942).
f. Grangaard, D. H., Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.,* **61**, 1290 (1939).
g. Haake, R. and W., U. S. Patent 1,792,088 (1931); German Patent [illegible]
h. Harvey E. H., U. S. Patent 1,726,824 (1923).
i. Hibbert, H., and Hiss, H. S., *J. Am. Chem. Soc.,* **45**, 734 (1923).
j. Hockett, R. C., and Maynard, C. W., Jr., *J. Am. Chem. Soc.,* **61**, 2111 (1939).

k. Kantorowicz, J., U.S. Patent 1,207,177 (1916).
l. Kerr, R. W., U.S. Patent 2,108,862 (1938).
m. Kerr, R. W., U.S. Patent 2,268,215 (1941).
n. Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.*, **64**, 585 (1942).
o. Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.*, **64**, 589 (1942).
p. Nicolson, J., U.S. Patent 2,221,552 (1940); British Patent 534,503 (1941).
q. Oldham, J. W. H., and Rutherford, J. K., *J. Am. Chem. Soc.*, **54**, 366 (1932).
r. Pierson, G. G., U.S. Patent 1,989,150 (1935).
s. Chemische Fabrik Pyrgrof G.m.b.H. and Haller, German Patent 438,119 (1924).
t. Richardson, W. A., Higginbotham, R. S., and Farrow, F. D., *J. Textile Inst.*, **27**, 131T (1936).
u. Schopmeyer, H. H., and Felton, G. E., U.S. Patent 2,218,221 (1940).

F. Phosphoric Acid Derivatives:

a. Fernbach, A., *Compt. rend.*, **138**, 428 (1904).
b. Fouard, E., *Compt. rend.*, **144**, 501 (1907).
c. Northrop, J. H., and Nelson, J. M., *J. Am. Chem. Soc.*, **38**, 472 (1916).
d. Tryller, H., *Chem. Ztg.*, **44**, 833 (1920).

G. Miscellaneous:

a. Adalberto, F., *Rev. soc. brasil chim.*, **10**, 333 (1940).
b. Bechamp, A., *Rep. Pharm.*, **51**, 255 (1860).
c. Burkhard, C. A., and Degering, E. F., *Rayon Textile Monthly*, **23**, 676 (1942).
d. Dreyfus, H., French Patent 756,150 (1933).
e. Eastman Kodak Company, U.S. Patent 1,928,652 (1933).
f. Farbenindustrie, British Patent 302,191 (1927).
g. Fehling, H., *Rapport ann. prog. chim., Berzelius, Paris*, p. 342 (1847).
h. Harrison, W., British Patent 264,261 (1925).
i. Harrison, W., British Patent 286,331 (1926); 286,332 (1926).
j. Haworth, W. N., and Machemer, H., *J. Chem. Soc.*, 2372 (**1932**).
k. Meyer, K. H., Wertheim, M., and Bernfeld, P., *Helv. Chim. Acta*, **23**, 865 (1940).
l. Mossgraber, E., German Patent 564,881 (1931).
m. Patents, miscellaneous: Farbenindustrie, German Patent 492,319 (1926); Harrison, W., German Patent 497,240 (1927); Farbenindustrie, French Patent 640,174 (1927); British Patent 326,865 (1928); French Patent 656,861 (1928); Frank and Mienes, German Patent 575,349 (1939).
n. Pokorny, J., *Kunststoffe*, **17**, 31 (1927).
o. Pringsheim, H., and Langhans, A., *Ber.*, **45**, 2533 (1912).
p. Pringsheim, H., and Wolfsohn, K., *Ber.*, **57B**, 887 (1924).
q. Radley, J. A., Starch and its Derivatives, D. van Nostrand Co., New York, 93 (1940).
r. Reich, W. S., *Compt. rend.*, **195**, 1029 (1932).
s. Reilly, J., Wolter, R., and Donovan, P. P., *Sci. Proc. Roy. Dublin Soc.*, **19**, 467 (1930).
t. Reinhart, F. W., and Kline, G. M., *Ind. Eng. Chem.*, **31**, 1522 (1939).
u. Rundle, R. E., Daasch, L., and French, D., *J. Am. Chem. Soc.*, **66**, 130 (1944).
v. Rundle, R. E., and Edwards, F. C., *J. Am. Chem. Soc.*, **65**, 2200 (1943).
w. Scholten, W. A., French Patent 732,306 (1931).
x. Sprague, G. F., Brimhall, B., and Hixon, R. M., *J. Am. Soc. Agron.*, **35**, 817 1943).
y. Van Linge, R. A., Dutch Patent 44,502 (1938).
z. Wengraf, P., *J. Soc. Dyers Colourists*, **46**, 245 (1930).

CHAPTER XI

OXIDATION OF STARCH

BY J. M. NEWTON AND GEORGE T. PECKHAM, JR.

1. Introduction. The subject of starch oxidation is generally approached from two distinct view-points, that of the research chemist interested in the chemical constitution of starch, who uses oxidative techniques as keys to the intricacies of the starch molecule, and that of the industrial starch chemist who uses oxidative methods to modify starches for certain specific industrial uses. In both view-points there is a common interest in that the same starting material is used and a multiplicity of oxidizing agents is allowed to act under varying conditions. The difference lies in the fact that those interested in studies of the structure degrade the original starch to chemical entities of determinable molecular structure, while the industrial starch chemist is interested in relatively mild oxidative changes that will adapt starch products to special commercial uses. Both view-points deal with the same reaction or series of reactions, the first group of investigators being interested in the complete reaction and in the reaction products, while the second group is primarily interested in those phases of the reaction which apply in the manufacture of oxidized starch products.

The greater portion of the published work on the oxidation of starch comes from academic research laboratories primarily interested in the complete elucidation of the structure of the starch molecule, and as a result their extensive oxidative treatments yield relatively simple chemical compounds. Information dealing with the commercial oxidation of starch must, in general, be obtained from the patent literature. Such sources of information yield only a very limited knowledge of the chemical changes taking place in commercial oxidations. The science has not as yet caught up with the art. Recently, however, a greater scientific emphasis has been placed on studies dealing with starches oxidized under conditions comparable to commercial practices.

In addition to the true oxidation of starch, there are various chemical and biological oxidations which produce such compounds as gluconic, glucuronic, saccharic, citric, lactic, acetic, propionic, and butyric acids, carbon dioxide, acetone, etc., from starch. Because all such oxidations probably proceed through glucose, they are really oxidative products of glucose. Such oxidations are therefore considered to be beyond the scope of this discussion.

2. Historical. The literature on the oxidation of starch dates from Justus Liebig's observation in 1829 (1) that starch is slightly affected by prolonged action of chlorine or chlorous acid. Pelouze (2) in 1838 established oxalic acid as one of the products formed during the oxidation of starch with concentrated nitric acid. Less extensive oxidation by bromine water and silver oxide allowed Habermann in 1874 (3) to isolate and identify gluconic acid as a degradation product of starch. These early investigations were followed by studies on the oxidation of starch by calcium and potassium permanganate, potassium dichromate, hydrogen peroxide, hydrogen peroxide and ferric chloride, sodium peroxide, fluorine, manganese dioxide, perborates, persulfates, hypochlorites, oxygen, sodium *p*-toluenesulfonchloramide (Activin), etc. For extensive bibliographies of references dealing with the oxidation of starch by various chemicals see Walton (4) and Radley (5).

3. **Oxidation by Hydrogen Peroxide.** The early investigators began a rather extensive investigation of the use of H_2O_2 as a reagent for the oxidation of starch. Wurster in 1889 (6) reported that H_2O_2 at ordinary temperatures did not attack starch. By making the H_2O_2 solutions slightly alkaline with NH_3 and boiling, it was found that the starch liquefied, the liquefaction being accompanied by the evolution of CO_2 and O_2 (7). Soon thereafter, Syniewski (8) found that a soluble starch could be prepared by the action of Na_2O_2. The use of acids, alkalies, and salts as catalytic agents for the oxidation of starch by H_2O_2 was suggested by Fernbach and Wolff (9). A series of investigations on the catalysis of the action of peroxide on starch followed this report. Durieux reported (10) that H_2O_2 or $FeCl_3$ acting alone on starch had very little effect, but that a mixture of the two caused a marked saccharification. This catalytic effect of traces of $FeCl_3$ or $FeCl_2$ on the oxidation of starch by H_2O_2 was again substantiated by Dhar (11). Palit and Dhar (12-14) then extended the investigations of the catalytic effect of traces of metal ions to the oxidation of starch and other carbohydrates in alkaline solutions by the action of atmospheric oxygen. Their investigations demonstrate that starch is oxidized to CO_2 and H_2O by bubbling air through an alkaline suspension of the starch in which a small amount of $Fe(OH)_2$ is suspended. In the presence of sunlight, ZnO was found to act as a photosensitizer promoting the oxidation of starch and other carbohydrates (15). The work of Brown (16) indicates that the action of H_2O_2 on starch in the presence of ferrous ions does not result in an oxidation of the starch, but rather in the hydrolysis of the starch to dextrins and sugars, these products then being oxidized by the peroxide. The hydrolytic stage of the reaction is thus analogous to the amylolytic hydrolysis of starch by enzymes, differing only in that the products are further hydrolyzed and oxidized to give acids and aldehydes of low molecular weight. Brown suggests that the reaction is a true catalysis, the iron acting to transfer energy from the breakdown of H_2O_2 to the starch molecule, the activated starch molecule thus becoming reactive and easily decomposed.

4. Oxidation with Halogens. Oxidation of starch with halogens appears to give four different types of reactions.

a. Oxidation of Aldehyde Groups to Carboxyl Groups. On the basis of the known behavior of starch degradation products under similar conditions it seems logical to believe that oxidation of aldehyde does occur in starch. The oxidation of aldohexoses to the corresponding acids by the action of the alkaline salts of hypo-halogen acids is the basis of the Willstätter-Schudel titration. The quantitative aspects of this oxidation have been widely utilized, one of the more recent being the use of hypoiodite in methanol to oxidize aldomonosaccharides to the corresponding aldonic acids for characterization as benzimidazole derivatives (17). The same fundamental reaction has been utilized for determining the chain length of dextrins produced by the action of α-amylase (18) and of hydrochloric acid on corn starch (19). In both cases the dextrins were quantitatively oxidized by alkaline hypoiodite, and the potassium salts of the dextrinic acids were isolated and analyzed for potassium content. Chain lengths calculated from the iodine consumed in the oxidation and the potassium content of the resulting salt were in excellent agreement. Samec (20, 21), Rassow and Lobenstein (22), and Samec and Blinc (23) concluded that the acid groups formed during mild oxidation of starch are derived from the aldehyde groups. The more recent work of Felton *et al.* (24) on the oxidation of starch with Br_2 in the presence of excess $CaCO_3$ indicates that non-uronic acids are formed.

b. Oxidation of Primary Alcohol Groups to Carboxyl Groups. Syniewski (25) isolated an "amylodextrinic acid" by the oxidation of amylodextrin with bromine. This product contained glucuronic acid, since the material gave a positive naphthoresorcinol test and yielded about 5.25% furfural upon distillation with HCl (this corresponds to 34.5% glucuronic acid anhydride). More recent studies (24) indicate that as much as 50% of the bromine-oxidized starch may consist of glucuronic acid anhydride. The first isolation of glucuronic acid from a starch product was reported by Farley and Hixon (26). The cinchonine salt of the acid was crystallized from samples of the oxidized starch hydrolyzed by sulfuric acid. Several recrystallizations gave the pure cinchonine salt of glucuronic acid, m.p. 199-200°C.*

c. Oxidation of Secondary Alcohol Groups to Ketone Groups. Everett and Sheppard (27) have made quantitative studies on the oxidation of more than fifty carbohydrates by bromine in acid solutions. Their data on the analyses of the oxidation products of starch indicated that considerable oxidation occurs on the secondary alcohol group. The presence of considerable reducing power against Fehling's solution at certain stages of oxidation is also indicative of ketone formation (24). Further evidence of the oxidation of secondary alcohol groups to ketones was shown by the formation of an oxime of oxidized starch (26). The nitrogen content of the oxime was found to be equivalent to one ketone group in 65 to 75% of the glucose anhydride units.

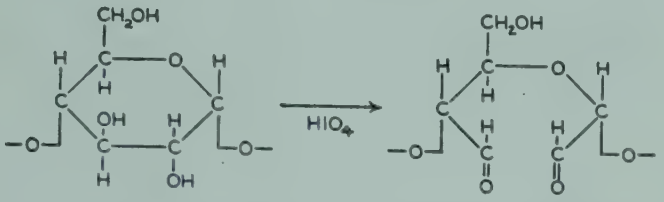
---

*Kerr has shown (Action of Nitrogen Dioxide on Starch and Its Fractions, in press, J. Am. Chem. Soc.) that NO_2 acts almost exclusively and quantitatively on the primary alcohol groups in starch to form anhydro glucuronic acid units and accordingly, the reaction may be used to demonstrate a difference in structure between linear amylose and amylopectin which is branched through 6-carbon hydroxyls.

d. Oxidation of Glycol Groups to Carboxyl Groups. The oxidation of the glycol group is of the same type as the specific oxidative action of periodic acid. The hydroxyl groups on carbon atoms 2 and 3 in the anhydroglucose nucleus function as a glycol unit. This group is first attacked by the oxidizing agent to convert the hydroxyl groups to aldehyde units, the carbon chain in the pyranose ring being ruptured between carbon atoms 2 and 3. Continued action of the oxidizing agent then oxidizes the aldehyde groups to the corresponding carboxylic acid groups. That such an oxidation occurs in starch is indicated by the decrease in optical rotation and the increase in calcium content of the acid salts formed by oxidation with bromine. Such a degradation is further supported by the separation of barium salts of dibasic acids containing less than 6 carbon atoms (26).

It should be recognized that all of these four types of oxidation may occur simultaneously during the oxidation of starch by halogens. The halogens appear to lack the specificity required for any one type of oxidation in such a polyfunctional molecule as the anhydroglucose unit.

5. Oxidation by Periodic Acid. In contrast to the more or less random oxidation by the halogens in general, oxidation by periodic acid is characterized by a high degree of specificity. The use of periodic acid for the oxidation of simple sugars was first used by Malaprade (28) in a study of the ring structure of various methyl aldohexosides. Further use of the same oxidizing agent was made by Karrer and Pfaehler (29) and Herissey *et al.* (30) in studies of the heterocyclic ring structure of hexoses and the alkyl hexosides. On the basis of the experimental evidence obtained in these investigations, Herissey and coauthors suggested that the hexose molecule is oxidized to a dialdehyde with the formation of 1 molecule of formaldehyde. Soon thereafter Jackson and Hudson published their investigations on the determination of the ring structure and α and β configuration of glycosides (31, 32). Oxidation of four of the eight possible methyl-α-D-aldohexopyranosides by barium hypobromite yielded the same dialdehyde and the same crystaline strontium salt. This isolation of the dialdehyde substantiated the theory of Herissey *et al.* (30) as to the mechanism of the oxidation of hexoses.



I. Hexose unit of starch II. Corresponding dialdehyde

Since starch consists of hexose units bound together in some chemical pattern, it would seem logical to apply the method of oxidation by periodic acid to starch. If we assume that starch molecules consist of hexose units having the generally accepted structures, then oxidation by periodic acid should yield substances similar to those obtained from oxidation of the simple hexose sugars.

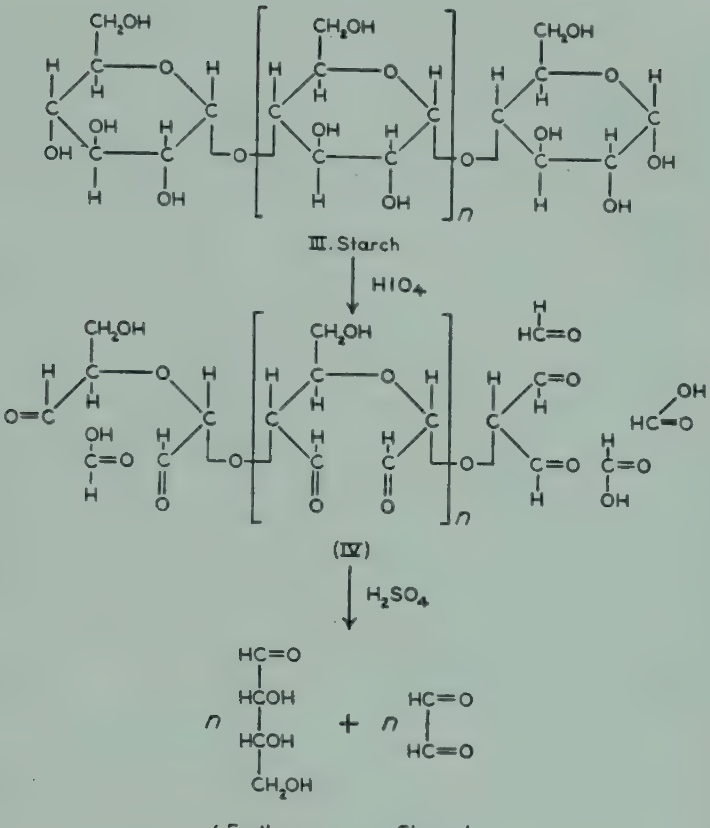
The first report of experimental oxidations of corn starch by periodic acid is that of Jackson and Hudson (33). The oxidation is carried out by suspending ungelatinized corn starch in an excess of 0.58 M aqueous HIO_4 solution and allowing the reaction to proceed at room temperature. At the end of 24 hours the quantity of oxidant consumed closely approximates one molecular equivalent, which is the theoretical amount required to oxidize the hexose unit of starch (I) to the corresponding dialdehyde (II). Since the consumption of HIO_4 proceeds at a greatly diminished rate after the consumption of 1 mole of oxidizing agent, it is apparent that the principal reaction is completed at this stage. This treatment of corn starch results in a quantitative yield of the oxidized product, the appearance of which is much like that of the parent corn starch when viewed under the ordinary microscope. However, when viewed between crossed nicols, the oxidized granules show a uniformly dark field in contrast to the characteristic cross of untreated starch granules. This loss of birefringence in the granules is readily followed by placing a few granules of corn starch in a drop of approximately 0.5 M HIO_4 and observing them between crossed nicols. The dark bands of the polarization crosses gradually spread and within a few minutes completely disappear.

The product obtained by the mole-for-mole periodic acid oxidation of corn starch is insoluble in cold water, soluble in hot water, gives no color reaction with iodine, reduces Fehling's solution, forms an amorphous precipitate with phenylhydrazine at 25°C., is not attacked by malt diastase, and has a specific rotation of about +9° at 20°C. in sodium light. If a thin layer of an aqueous solution of the oxystarch is poured into a glass plate and allowed to dry, a clear transparent film can be produced. Hydrolysis by boiling 0.1 *N* aqueous HCl gives a solution with a levo equilibrium rotation of −13 to −16°. Because this rotation approximates that reported for D-erythrose, and because hydroylsis of the proposed dialdehyde structure would be expected to yield D-erythrose, Jackson and Hudson suggested that the main optically active component of oxystarch hydrolysates might be D-erythrose.

Working concurrently, but independently, Caldwell and Hixon (34) and Jackson and Hudson (35) carried on further investigations relative to the oxidation of starch by periodic acid. These investigations have materially assisted in the elucidation of the mechanism of oxidation by periodic acid. On the basis of fairly well accepted concepts, the starch molecule consists of a chain of glucopyranose units joined by 1, 4-α-glucoside linkages (36). The theoretical oxidation of such a molecule by periodic acid would proceed as shown in the first two steps of the following reaction. The intermediate glucose units would be oxidized as reported by Jackson and Hudson (33), while the terminal units would give rise to 3 molecules of formic acid (28, 30) and 1 molecule of formaldehyde. Acid hydrolysis of the oxidized starch should yield glyoxal and D-erythrose as the principal products and certain other decomposition products in small amounts. This hydrolytic reaction is shown in the final step of the following reaction. (See p. 331.)

By assuming this theoretical oxidation to be correct, it is evident that the quantitative determination of the amount of formaldehyde formed during oxidation of the starch by periodic acid should furnish a method for measuring molecule size. Caldwell and Hixon (34) utilized this method for the measurement of the molecular size of starches and dextrins. By comparing the chain length of the various dextrins as calculated from the experimentally determined amount of formaldehyde with the chain length of the same fraction as calculated from the reducing value (37), satisfactory correlations were obtained. The analytical data obtained from oxidations by periodic acid therefore suggest that the chain length of the fundamental starch unit is much longer than the 25 glucose units obtained by Haworth *et al.* (38) from their methylation data.

The first characterization of the products obtained by the acid hydrolysis of periodate-oxidized corn starch was the isolation and identification of glyoxal by preparation of the osazone and benzylphenylosazone (34). The osazone crystallizes as light yellow, elongated, hexagonal plates with a melting point of 172-175°; the benzylphenylosazone crystallizes as fine white needles melting sharply at 195°. This isolation of glyoxal serves to substantiate the theoretical equation for the oxidation of the non-terminal glucose units of starch by periodic acid as written above. Identification of D-erythrose, the



III. Starch

(IV)

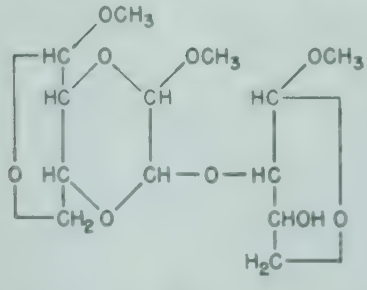
d-Erythrose Glyoxal

remaining hydrolytic product in the proposed mechanism, would furnish conclusive proof for the rupture of the non-terminal glucose units between carbon atoms 2 and 3. Caldwell and Hixon were unable to identify D-erythrose in the hydrolytic products. However, they report that a private communication from Jackson and Hudson informed them that, in addition to glyoxal, D-erythrose has been identified in the hydrolytic cleavage products of periodic acid oxystarch (34). The presence of glyoxal interferes with the isolation and identification of D-erythrose. By treating the hydrolysis products of periodic acid oxystarch with bromine water, the glyoxal can be removed as an interfering reactant by oxidation to oxalic acid and precipitation as the barium salt. The *d*-erythronic acid is then crystallized as brucine *d*-erythronate, m.p. 211° (with decomposition). *d*-Erythronic lactone, m.p. 104-105°, can be prepared from the pure brucine *d*-erythronate (35). These results prove that periodic acid breaks the carbon chain of the non-terminal glucose units of the starch molcule between carbon atoms 2 and 3. They also offer confirmatory evidence for the generally accepted structure of the predominating units in starch.

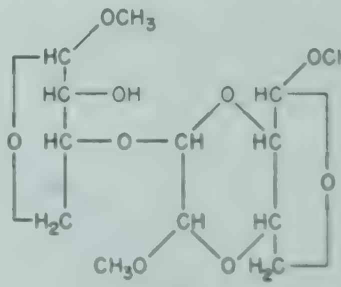
The oxidation of starch by periodic acid gives the same results whether the oxidation is carried out on the raw granules or on gelatinized starch. Jackson and Hudson have always applied the oxidizing agent to the whole starch grains, while Caldwell and Hixon applied periodic acid to gelatinized starch pastes. Their results agree in all respects. When periodic acid was applied to soluble starch and insoluble powdered starch, by the same investigators, the rate curves for periodate ion consumption, for both starches were found to be practically superimposable (39). It is thus evident that the periodate ion is capable of penetrating into the starch granule and simultaneously attacking all parts of the micellar structure.

Further studies on periodate-oxidized starches show that the application of methanolysis to the oxystarch yields small quantities of a crystalline substance of unknown composition (40, 41). The material is a white, crystalline, levorotatory compound melting at 148°, and obtainable in yields up to 2%. It is non-reducing, but after mild acid hydrolysis it reduces Fehling's solution and appears to be the methyl acetal of an aldehyde or ketone. The same crystalline product is obtained from periodate-oxidized corn, wheat, potato, arrowroot, and soluble potato starch.

The excellent research of Michel and Purves (42) has given considerable information as to the nature of this crystalline compound. Their work shows that the compound has the empirical formula $C_{10}H_{12}O_5(OH)(OCH_3)_3$, that all five oxygen atoms in the saturated aliphatic nucleus are centers of ether or acetal linkages, and that three cyclic ring systems are probably present. Remembering that the compound is derived from periodate-oxidized starch (II), which consists of repeating dialdehyde units containing one erythrose and one glyoxal residue, it becomes possible to form two structures which would satisfy the experimental evidence. These structures vary only in the point of attachment of the glycosidic bond. Michell and Purves thus propose that the crystalline substance isolated from methanolysis of periodate-oxidized corn starch consists of a methylerythrofuranoside unit combined in



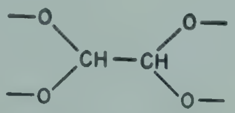
(V)



(VI)

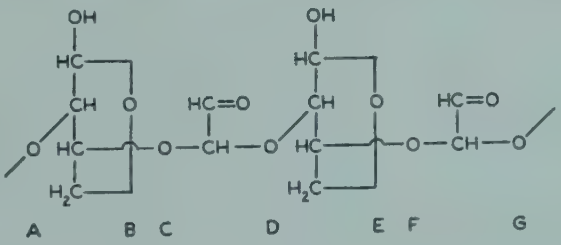
a 1,4-dioxane ring with a glyoxal residue, to which a second methylerythrofuranoside residue and a methyl group are separately attached through glyoxal hemiacetal linkages. Structures (V) and (VI) satisfy these requirements.

Additional experimental evidence to support the proposed structures is furnished by further methanolysis of the crystalline compound under drastic conditions (42). Steam distillation of the products of methanolysis gives a good yield of glyoxal tetramethylacetal. This provides evidence for the presence of the unit in the crystals.



The *p*-toluenesulfonyl monoester of the crytalline compound derived from the oxystarch is prepared by the action of *p*-toluenesulfonyl chloride in pyridine. The formation of the monoester supports the presence of only one hydroxyl group. Treatment of this monoester with pure sodium iodide in dry acetone under pressure at 100°C. for 2 hours, followed by recovery of the products, shows that about 85% of the original *p*-toluenesulfonate is recoverable in a pure crystalline form. Since the *p*-toluenesulfonyl group in derivatives of primary alcohols is known to be replaced quantitatively by iodine under these conditions (43), the recovery of the major portion of the original monoester shows that the crystalline oxystarch derivative does not contain a primary alcohol group.

The formation of such ring systems as those suggested for the methanolysis products of periodate oxystarch are at first difficult to comprehend. Their formation can best be explained by assuming that the erythrose portion of the dialdehyde oxystarch structure follows the normal tendency of erythrose to condense to a furanoid form (44). By assuming that the furanoid ring forms in every erythrose unit, we can write the structure of starch oxidized by 1 mole of periodic acid, as shown in (VII). The glyoxal acetal bonds,

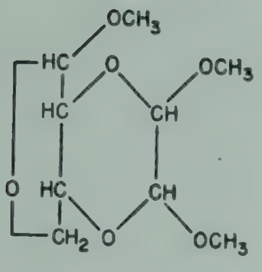


(VII)

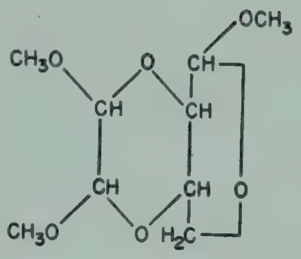
A, D, G, etc., are the glycosidic bonds of the original starch; the furanoid bonds, B, E, etc., are formed by the normal cyclization of the erythrose units; and C, F, etc. are the pyranoside rings in the original starch. Methanolysis of such a structure with hydrogen chloride in methanol would cleave the glyoxal acetal bonds and the original pyranoside linkages, but would have no effect on the newly formed furanoside rings. Each cleavage would produce a new hydroxyl group on either the second or third carbon of the erythrose residue, and the condensation of this newly formed hydroxyl group with the free aldehyde group in the adjacent glyoxal unit would result in a substituted 1, 4-dioxane ring. The probability of such a hemiacetal condensation is supported by similar cyclizations between known molecules of a similar structure. The formation of (V) can then be explained by assuming that methanolysis first occurs at A, followed by cyclization of the newly formed hydroxyl group on carbon atom 2 of the glyoxal residue, then methylation of the hemiacetal hydroxyl groups, and finally a rupture at F. If the initial hydrolysis had occurred at F and the final methanolysis at A, structure (VI) would be produced. Since bond A is glycosidic in nature and bond G pyranosidic, methanolysis of the respective linkages might not proceed at the same rate. This would give rise to unequal amounts of (V) and (VI).

An additional complicating factor is the fact that formulas (V) and (VI) should each give rise to eight isomeric forms depending on the various possible α and β configurations of the three methoxyl groups. The crystalline material isolated from the methanolysis of the periodate-oxidized starch is believed to be one of these isomers in a pure state. The mother liquor from this crystallization was carefully examined by Michell and Purves (45), and their experimental evidence indicates that the material is structurally identical to the crystalline fraction. This entire fraction must, therefore, consist of two or more isomers of structures (V) and (VI). The structural identity of these two fractions gives a total yield of 22% of these isomeric hexahydro-3, 5-dimethoxy-2-(1-methyl-3, or 2-erythrofuranosyloxy)furo-[3,4]-*p*-dioxins. The exact identification of the isomeric forms present in the methanolic degradation products of periodate oxystarch will serve as future problems for the research chemist.

Since materials of structure (V) and (VI) account for less than 25% of the total oxidized starch, Michell and Purves (45) have extended their studies to other fractions obtained in the methanolysis of periodate oxystarch. Fractional distillation of the methanolic cleavage products gives a mobile, colorless material, which distils at 116-119°C. at 5 mm. pressure, sp.gr. (20°/20°) of 1.204, $n_D^{20} = 1.4488$, and a molecular weight of 226 in CCl_4. An ether + petroleum ether solution of the fraction on standing gives a white, bulky precipitate. Recrystallization yields a pure crystalline substance, m.p. 97-98° $[\alpha]_D^{20} = -59.1°$ and a molecular weight of 212 in CCl_4. On the basis of their studies Michell and Purves believe the entire fraction to be a mixture of isomeric hexahydro-2,3,5-trimethoxyfuro-[3,4]-*p*-dioxins (VIII) and (IX), one of which crystallizes as described above



(VIII)

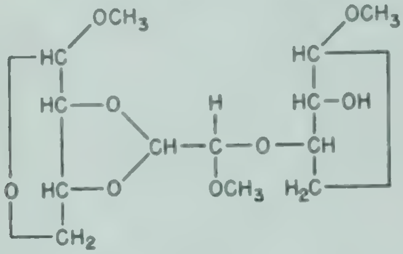


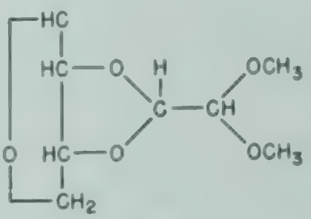
(IX)

The entire fraction accounts for another 18% of the original oxystarch. The formation of such structures by methanolysis of oxystarch (VII) would require two types of bond rupture. Cleavage of oxystarch at bonds A, D, G, etc., followed by condensation and methylation, would give rise to (VIII), while cleavage at C, F, etc. would give rise to (IX). Structures (VIII) and (IX) can also be derived by removing the erythrose residue from formulas (V) and (VI). This degradation has been demonstrated by Michell and Purves (45). Treatment of a mixture of the isomeric forms of (V) and (VI) with a 10% solution of hydrogen chloride in dry methanol gives a 36% yield of a fraction which has properties identical to those of mixtures of the isomeric forms of (VIII) and (IX). Analytical data for the two fractions give additional proof of their identity.

Continued efforts to identify erythrose in a hydrochloric acid hydrolysate of (V) and (VI) have been unsuccessful. Analytical data suggest that the glyoxal and erythrose which initially form under hydrolytic conditions immediately recombine under the influence of aqueous hydrochloric acid to give unidentified condensation products. That cyclic acetals can be formed under similar conditions is demonstrated by the acetal condensation of bromoacetaldehyde and mannitol (46). A more striking example is the formation of 2,3-ethylenedioxydioxane in 20% yields by the condensation of glycol and glyoxal tetramethylacetal in normal aqueous or aqueous alcoholic hydrogen chloride (47). In the last example, condensation to give a cyclic acetal occurs under conditions which supposedly are ideal for hydrolysis of such compounds. Therefore, the hydrolytic conditions applied to (V) and (VI) in an attempt to form erythrose and glyoxal may be ideal for their primary formation, yet these same conditions may also be ideal for their recondensation into other cyclic acetals. This would explain the failure to isolate erythrose as a hydrolytic product.

The possibility of recondensation of hydrolytic products introduces an added factor in the study of the methanol + hydrogen chloride breakdown products of periodate oxystarch. If we assume that methanolysis should break every bond in (VII), we would have a mixture of erythrose and glyoxal, or their methyl derivatives. If a reversion process then set in, structures (V), (VI), (VIII), and (IX) could be formed by condensation of


(X)


(XI)

the glyoxal and erythrose. Under these conditions the products of the methanolysis might not have the 1,4-dioxane structures tentatively assigned to them but, rather, be cyclic acetals of structures (X) and (XI). It seems improbable that structures (V), (VI), (VIII), and (IX) could be produced in the observed yields by reversion processes. However, as pointed out by Michell and Purves (45), the possibility of a recondensation of the initial scission products must be simultaneously considered along with cyclization of carbonyl with conveniently situated hydroxyl groups as an explanation of the formation of the methanolic degradation products of periodate oxystarch.

The quantitative determination of the glyoxal units present in oxidized starches serves as a means for the determination of the dialdehyde type of oxidation in periodate oxystarches (39). Methanolysis with 10% hydrogen chloride in methanol yields glyoxal tetramethylacetal and the 1,4-dioxane derivatives discussed previously. Simple steam distillation of the products of samples of known composition, followed by determination of the distilled glyoxal by precipitation with 2,4-dinitrophenylhydrazine, shows that only about one-half of the expected glyoxal residues is distilled. Studies on the distillation rates of the products known to be present have shown that the cyclic, 1-4-dioxane derivatives do not distil under ordinary steam distillation techniques. However, if the aqueous methanolysis products are steam-distilled nearly to dryness, additional water added, and steam distillation continued until the still pot is again nearly dry, over 90% of the theoretical glyoxal residues is steam-distilled. This method has been carefully studied by Grangaard *et al.* (39) and, under the conditions which they have established, at least 90% of the glyoxal units theoretically present in periodate oxystarch can be recovered. This gives us an analytical tool for the study of starch oxidations in which dialdehyde structures are formed. The analytical method is also applicable to oxidations of cellulose.

6. **Oxidation by Commercial Methods.** The published literature con-

tains little information on the changes brought about by the commercial oxidation of starch. The closest approximation is given by Farley and Hixon in their report on the properties of starch oxidized by hypochlorite produced by the electrolysis of alkaline sodium chloride solutions (26). The changes during oxidation were followed by determinations of hot viscosity, rigidity, gel strength, reducing power, turbidity, volume of swollen granules, quantitative birefringence, digestibility by β-amylase, and microscopic observation of the swollen granules. Rigidity, gel strength, and turbidity decrease proportionally to the amount of oxidizing agent used. With low hypochlorite consumption no increase in reducing power is observed, but the more highly oxidized products show marked increases in reducing power. Oxidation decreases the amount of maltose produced by the action of soybean β-amylase. Photomicrographs of the starch granules oxidized with various quantities of hypochlorite and gelatinized in 0.70% NaOH show marked differences. With increased oxidation the granules become more resistant to swelling, until starch granules oxidized with 0.5 equivalent of chlorine per unit fail to swell. These highly oxidized granules start to disintegrate along the visible radial fissures, causing the granule to break into several fragments. These fragments then break into numerous small pieces and finally dissolve in the solution of sodium hydroxide. It is most interesting to note that this highly oxidized starch upon centrifuging gave an almost clear paste of very low viscosity and no sediment of swollen granules.

The experimental observation that the quantitative birefringence of starch granules does not change by oxidation with as much as 0.5 equivalent of chlorine per glucose unit suggests that the crystalline regions of the starch granule are not attacked by the oxidizing agent (26). However, swelling experiments show that these oxidizing agents penetrate deeply into the starch granules, causing marked changes in the rate and extent of granule swelling. On the basis of these experiments Farley and Hixon suggest that the oxidizing agent attacks the starch granules between radial starch crystallites. Solution of the oxidized portions would give rise to the cracks and fissures observed in oxidized starch granules. Upon continued reaction the oxidizing agent would continue to attack the non-crystalline portion of the starch, and thus penetrate the entire granule. Such a mechanism of attack would explain the partial solubilization of starch upon oxidation, the formation of cracks and fissures, and the characteristic "swelling" behavior of oxidized starch; further, this method of attack would allow the starch granule to retain its quantitative birefringence.

Commercially oxidized starch resembles raw starch in that it retains practically the same granule structure, shows typical polarization crosses, is insoluble in cold water, and shows the characteristic starch color reactions with iodine. However, when heated with water the unoxidized starch yields pastes or gels, while the oxidized starches at equal concentrations give thinner bodied solutions, the differences varying with the degree of oxidation. Because of the increased fluidity of the pastes the oxidized starches are commonly designated as "thin boiling starches." This term is also applied to acid-modified starches.

The degree of modification of oxidized starches is expressed in terms of fluidity. Fluidity refers to the volume of a suspension of pasted starch of specified concentration which will flow through a standardized orifice in a specified period of time as compared to a specific volume of water which has flowed through the same orifice in the same length of time. For example, a "60 fluidity" starch means that 60 cc. of the starch suspension (the concentration of the suspension varies with the type of starch used) have flowed from the standard orifice in the same period of time that 100 cc. of water would require.

Oxidized starch has (1) a shorter cooking time, (2) higher fluidity, (3) increased adhesiveness, (4) lower rate of congealing, and (5) gives a less turbid suspension than the parent starch. Films formed by drying oxidized starch pastes are of a tough and horny character in comparison with the extremely brittle films of unoxidized starch. These properties make oxidized starch particularly adaptable to the surface sizing of paper and to the sizing and finishing of textiles.

Raw cotton yarns, containing no size, do not have sufficient strength to withstand the stretch to which they are subjected on the loom if used as warps, and do not offer the proper resistance to abrasion. By sizing the warp threads with a proper mixture of starch, gums, lubricants, and softeners the yarn is conditioned to have the necessary stiffness, strength, elasticity, and pliability to withstand the mechanical wear and strain of weaving. The greater adhesiveness of the oxidized starches gives warps with individual fibers properly cemented together so as to form a strong, elastic, pliable thread with the proper amount of starch on the surface to protect the yarn from abrasive action. These oxidized starches cook without foaming in the kettle, require less gum and compound, flow through the pipe lines freely, remain at a constant fluidity with continued circulation, and drain out of the size kettle, storage tanks, and size box without leaving any hard size. In addition, the oxidized starches are exceptionally white and clean.

In paper making, starches and the highly oxidized starch gums are used to close the pores of the paper, to lay fuzz on the surface, to increase tensile, fold, and bursting strength (Mullen), and to give "feel" and rattle to the paper. The starch gums also act as protective colloids resulting in a finer, more uniform dispersion of the rosin size.

The most common agents used commercially for the oxidation of starch are sodium and calcium hypochlorite. For this reason many commercially oxidized starches have been referred to as "chlorinated starches." This is a misnomer, because the chlorine does not substitute into the starch molecule as the name infers, but rather serves to provide the oxidizing potential. This oxidation is brought about by the reduction of the positive valent chlorine in NaOCl to the negative valent chlorine in NaCl, with the subsequent release of ½ mole of oxygen.

The general commercial method for producing hypochlorite-oxidized starches consists of treating aqueous starch suspensions (about 36%) with a hypochlorite solution (6 to 8% active chlorine) containing a slight excess of alkali. The hypochlorite solution is added in small portions so that the heat generated can be dissipated by the cooling system, the general practice being to keep the temperature between 90 and 125°F. The heat produced is probably due to three exothermic reactions: (1) the heat of adsorption, (2) the heat of decomposition of the hypochlorite, and (3) the heat generated in oxidizing the starch (22). When the reaction is judged to be approaching the desired degree of oxidation, a sample of the starch milk is removed, filtered, and washed on a vacuum filter, and the fluidity of a paste of definite concentration prepared from the cake is then determined. When a product of the desired fluidity is obtained the oxidation is stopped by adding an antichlor (usually sodium bisulfite), and the slurry is adjusted to the desired pH (usually 3 to 7), filtered, washed, water removed to about a 47 to 50% moisture content, and dried. By varying the quantity of hypochlorite used and the time, temperature, and pH of the reaction, an entire series of oxidized starches can be produced.

In addition to the wet process for producing hypochlorite-oxidized starch, some use is made of a dry process. In this process completely dried starch is put into a mixer provided with a water jacket. A solution of sodium hypochlorite containing 8% of available chlorine and 2% of sodium hydroxide is sprayed on the continuously agitated starch. The rate of addition of hypochlorite and the amount of cooling water flowing through the jacket are adjusted so that the maximum temperature is maintained at 45°C. (113°F). At the end of the reaction the product usually contains about 32% moisture, which is then reduced in any suitable drier to 10 to 20% (48, 49).

Nearly all commercially oxidized starches are thinner boiling and have less tendency to congeal than the parent starches. However, it is possible to prepare starches with increased fluidity by treating with hypohalites under properly controlled conditions. For example, Bryant (50) describes a process for increasing the viscosity of starch products in which very small amounts of sodium hypochlorite are added to the starch slurry before the final removal of water. The amount of hypochloride added is just slightly in excess of that amount calculated to oxidize the sulfur dioxide present in the starch Thus, the function of the oxidizing agent appears to be the removal of the sulfur dioxide, which may act as a modifying agent in the subsequent pasting operation.

The oxidation of starch by halogens in slightly acid solutions to produce starches with maximum hot paste viscosity and low cold paste viscosity has been patented by Kerr (51). This patent is based upon the discovery that when starch is treated with a halogen, in properly regulated amounts and at temperatures of about 125°F., the viscosity curve of the hot pastes rises very sharply during the first part of the reaction and then declines to give typical "thin boiling starch" viscosities. A similar rise in viscosity in the early phases of starch oxidation by electrolytic hypochlorite in alkaline sodium chloride solutions is reported by Farley and Hixon (26). Their work shows that with increasing degrees of oxidation the viscosity of starches increases to a maximum at 0.2 equivalent of chlorine per anhydroglucose unit, and then decreases with additional oxidation.

Another interesting halogen-oxidized starch is described by Kerr (51). By treating a 30% starch slurry with chlorine or bromine (preferably bromine) at 75°F., a thick-boiling, thick-setting starch can be produced. These thick-boiling, thick-setting starches are capable of adsorbing more water than the parent starch from which they were prepared. By following this oxidation with an acid treatment, a product can be obtained the paste of which is less congealing than a product of the same fluidity made by hydrolyzing raw corn starch with acid alone.

Oxidation of dry starch by chlorine gas also yields products whose pastes have viscosities lower than those of the parent starch (52). The patents covering this process claim that "by varying the temperature of treatment the length of the treatment, which may vary from one to twelve hours, and the amount of chlorine used, it is possible to produce any modified starch product ranging from thin boiling starch, substantially insoluble in cold water (1% soluble) to dextrines which have solubilities in cold water of over 90%."

The oxidation with hypochlorite is also utilized in the manufacture of refined sago starch products from crude sago flour (53). From 0.25 to 0.6% of available chlorine, based on the dry weight of the starch, is added to aqueous suspension containing 25 to 30% of starch. The available chlori is always added as an alkaline hypochlorite solution, preferably as calcium pH value may be used, particularly when a non-cereal starch is used such as tapioca, which gelatinizes more readily than corn starch. However, in so doing, one runs the risk of allowing greater activity of the saccharogenic factor, and the unrestricted production of sugars detracts from the strength of the dextrin produced. β-Amylase has a peak activity between 45-50°C., at pH between 4.5 and 5.0.

Adhesive manufacturers have studied this process in detail and have developed variations to produce special effects or to obtain economies in operation. Greater enzyme activity may be obtained and possibly higher starch to water ratios may be used, if the enzyme is added progressively in small portions as the heating proceeds and if the increase in the temperature takes place slowly. High starch concentrations are desirable, since certain manufacturers partially dehydrate the final product by evaporation. The more water present, the more costly is the process.

An extreme case was studied by the writer. An attempt was made to convert a starch slurry made of 1 part of starch to 2 parts of water. In order to avoid the production of an unmanageable plastic mass in the early stages of conversion, due to a gelatinization of unconverted starch, it became necessary to extend the preliminary heating period over several hours time. The enzyme was added progressively in five equal portions. One addition an hour was made at 100, 125, 135, 145, and finally at about 155°F, when it appeared that the conversion was in the last stages. The surprising result was obtained that the dextrin had very little strength and that sugar production was excessive. It was subsequently found and reported (50) that in a delayed treatment of starch at temperatures below the commonly accepted gelatinization point the soluble end-products are almost exclusively highly fermentable sugars.

Dextrins which tend to revert less and remain free flowing are produced from starch according to an elaborate modification described by Singer (51). Starch is mixed with water at a concentration of 25% (1 part of starch to 3 parts of water) and heated to 149°F. as rapidly as possible. A quantity of malt extract (4.5% based on the weight of starch) is added. The malt extract has a diastatic strength of only 75° Lintner. The temperature is rapidly raised to 158°F. and then raised about 2°F. per minute until 176°F. is reached. The mass is then brought to boiling quickly. The conversion mixture is heated under pressure at 280°F. for 5 minutes. It is cooled to 176°F., and 18% more malt extract is added. The temperature is held at 176°F. for 25 minutes, whereupon the solution is again brought to boiling temperature. A very thin dextrin results, suitable for such uses as a remoistening gum.

As with the other types of dextrins described, various plasticizing agents, retrogradation retardants, and colloid modifiers may be added to or incorporated in the final product of enzymic dextrinization. Representatives of these modifying agents are urea, dimethylol urea (52, 53), dicyandiamide (54), hexalin, borax, and calcium chloride.

From the discussion it should be apparent that the type of conversion process used to produce dextrins by enzymes differs in important details from the diastatic conversion of starch employed by the fermentation industries. The two processes should not be confused. Attempts to employ practices found in the literature for the latter, such as the use of various enzyme accelerators or stabilizers or the use of a more acid conversion medium may lead to disappointing results.

Special equipment or improvement in the design of equipment used in the various steps of dextrin manufacture, in addition to those already discussed, is described in several patents (3, 55-60).

In a recent patent, Gardner (61) claimed a process for heating dry starch with a primary alcohol, to which acid may be added as a catalyst. The superior colloidal properties reported for these products may arise from a minor amount of etherification and if this is the case, a bridge is provided connecting the technology of derivatization, the chemistry of which is discussed in Chapter X, with dextrin manufacture. In any event, it would appear that a new phase of dextrinization will assume a position of increasing importance in the future as the industry makes use, more and more, of chemical derivatization reactions which may be applied to dry starch at higher temperatures.

BIBLIOGRAPHY

1. Frahm, H., *Ber.*, **74**, 622 (1941). See also, Dimler, R. J., Davis, H. A., and Hilbert, G. E., *J. Am. Chem. Soc.*, 68, 1377 (1946).
2. Brimhall, B., Dissertation, Iowa State College, Ames, 1942.
3. Krause, German Patent 549,711.
4. Henkel and Cie, G.m.b.H., German Patent 140,488 (1934).
5. Bloede, V. G., U. S. Patent 1,268,672 (1918).
6. Wakeman, L. K., U. S. Patent 2,126,520 (1938).
7. Bauer, H. F., Bauer, J. V., and Hawley, D. M., U. S. Patent 2,188,329 (1940).
8. Dextrin Automat, Limited, Australian Patent 99,013 (1924).
9. Dextrin Automat, Limited, French Patent 575,985 (1924).
10. Wulkan, H., U. S. Patent 1,139,621 (1915).
11. Wilson, G. F., and O'Reilly, P., U. S. Patent 248,904 (1881).
12. Verity, J., U. S. Patent 1,016,803 (1912).
13. Henkel and Cie., German Patent App., H. 133,619 (1932).
14. Horesi, A. C., U. S. Patent 2,274,789 (1942).
15. Bode, H. E., U. S. Patent 2,156,488 (1939).
16. Berlin, H., *J. Am. Chem. Soc.*, **48**, 1107 (1926).

17. Pochin, H. D., and Wolley, J., British Patent 1,544 (1857).
18. Bulfer, A. J., and Gapen, C. C., U. S. Patent 2,287,599 (1942).
19. O'Neill, C., British Patent 1,861 (1858).
20. Bloede, V. G., U. S. Patent 536,260 (1895).
21. Jones, W. L., U. S. Patent 2,045,988 (1936).
22. Kerr, R. W., U. S. Patent 2,108,862 (1938).
23. Berquist, C., U. S. Patent 1,851,749 (1932).
24. Fuller, A. D., British Patent 383,778 (1932).
25. Fuller, A. D., U. S. Patent 1,937,752 (1932).
26. Fuller, A. D., U. S. Patent 1,942,544 (1934).
27. Pinel, J. F., British Patent 10,648 (1845).
28. Maltby, W., British Patent 1,050 (1860).
29. Bloede, V. G., U. S. Patent 51,991 (1867).
30. Stutzke, R. W. G., U. S. Patent 1,320,719 (1919).
31. Browning, W., and Barlow, J. J., U. S. Patent 773,469 (1904).
32. Fielding, A., British Patent 20,488 (1907).
33. Fielding, A., British Patent 22,455 (1910). See also, Morris, S. G., U. S. Patent 2,359,378 (1944).
34. Lenders, A. W. H., U. S. Patent 1,159,592 (1915).
35. Wulkan, H., U. S. Patent 993,011 (1911).
36. Edson, E. R., U. S. Patent 1,956,064 (1934).
37. Fuller, A., U. S. Patent 2,014,799 (1935).
38. Stein, Hall Mfg. Co., British Patent 429,833 (1935).
39. Meyer, C. H., U. S. Patent 742,469 (1903).
40. Stutzke, R. W. G., U. S. Patent 1,516,512 (1924).
41. Bruce, T. A., U. S. Patent 2,070,253 (1937).
42. Bruce, T. A., British Patent 442,398 (1936).
43. Mathiessen, C. H., and Krieger, J., U. S. Patent 382,368 (1888).
44. I. G. Farbenindustrie Aktiengesellschaft, French Patent 852,033 (1939).
45. Coppock, P. D., U. S. Patent 2,181,782 (1939).
46. Clegg, J. E., and Hilliard, F. C., U. S. Patent 2,127,205 (1938).
47. Caesar, G. V., U. S. Patent 2,131,724 (1938); U. S. Reissue 21,057 (1939).
48. Haake, R., and Haake, W., U. S. Patent 1,792,088 (1931).
49. Lenders, A. W. H., U. S. Patent 1,305,291 (1919).
50. Kerr, R. W., Meisel, H., and Schink, N., *Ind. Eng. Chem.*, **34**, 1232 (1942).
51. Singer, P. A., U S Patent 2,175,486 (1939).
52. Glarum, S. N., and Thomas, J. J., U. S. Patent 2,302,309 (1942).
53. Glarum, S. N., and Thomas, J. J., U. S. Patent 2,302,310 (1942).
54. Kunze, W. G., and Evans, R. B., U. S. Patent 2,282,364 (1942).
55. Merrill, J. J., U. S. Patents 1,425,497 nd 1,428,604 (1922).
56. Brindle, R. G., U. S. Patent 1,505,696 (1924).
57. Darrah, W. A., U. S. Patent 1,689,379 (1928).
58. Dreyer, A., and Kutzer, F., German Patent 62,646 (1932).
59. Phillips, N. C., U. S. Patent 1,894,570 (1933).
60. Amthor, O., German Patent 79,212 (1938).
61. Gardner, H. F., U. S. Patent 2,374,676 (1945).

CHAPTER XIV

ACID HYDROLYSIS OF STARCH

1. Introduction. The discovery that starch will yield sugar-like substances when treated with acid is apparently attributed to the Russian chemist, Kirchoff (*1*). His experiments, reported in 1811, established the basis for the commercial production of starch sirups and the crude starch sugars. That the reaction is a hydrolysis and that the end-product from starch is identical to grape sugar or glucose were determined a short time later by de Saussure (*2*). The refined sugar is referred to as dextrose in American practice.

Attempts to study the mechanism of the reaction have been complicated mainly by three factors: failure to recognize and appreciate the importance of secondary reactions; lack of suitable analytical methods to study the system of reactions; and a lack of knowledge concerning the chemical nature of the substrate, starch. The result is that most of the early work, in common with many other phases of starch chemistry, is of little value and is of interest mainly to the historian.

2. Starch As the Substrate in Hydrolysis. The early attempts to determine the chemical composition of the starches and the constitution of starch components have been discussed in a previous chapter. The confusion which has prevailed during the past century in respect to this subject has been indicated. Very considerable progress has been made within the last decade, however, in that the principal problems involved have apparently been solved. It has been established that the starches, in general, are composed of two molecular species, although the proportion of each no doubt varies from starch to starch, depending on its biological origin. The component species occurring in smaller amounts (and lacking in several starches) consists of chains of 1-4 α-glucosidically linked glucopyranose units. These species are found to vary in length from possibly 100 to 1000 glucopyranose units. The balance of starch has a more complex structure which is probably of much greater molecular weight. In addition to the maltose type of linkage, evidence points to branching or ramification through 1-6 α-glucosidic linkages. Estimates of the molecular magnitude of such giant molecules and a determination of their exact structure are extremely difficult. It would seem likely that the order of magnitude for molecular weight averages about DP 1500 to 3000, or possibly more. That the pattern of the more complex fraction varies from starch to starch would seem to be settled. Whether this pattern varies within a starch, that is, whether varying degrees of branching exist so that any one starch may be considered to be composed of a series of molecular species with increasingly complex structures, or whether the variation in behavior noted for the two major species of a given starch is of molecular magnitude only, is an unsettled question. For the sake of discussion, however, that part of starch which is of lower molecular magnitude and which behaves as though it were a chain structure will be referred to as the "linear fraction." The balance will be referred to as the "branched fraction." The former also may be called amylose and the latter amylopectin, although it should be borne in mind that this usage does not necessarily correspond to the varied use which has been made of these terms to denote starch fractions during the last 40 years.

It has been established that all starches are not entirely composed of carbohydrate. Some starches, such as potato, contain molecules of amylopectin which are esterified with phosphoric acid. Other starches, such as corn, contain non-carbohydrate residues, *e.g.* fatty acids, so closely associated with the carbohydrate that they cannot be removed from the latter in the usual process of starch manufacture or in the laboratory with the common solvents for such substances. Corn starch, with the hydrolysis of which this chapter is chiefly concerned, is composed of about 30% amylose. At least a third of this amylose is undisputably linear in structure. The amylopectin is probably phosphate-free but the whole starch has associated with it, no doubt by adsorption, inorganic phosphates, the cation of which varies according to the starch manufacturing process. Fatty acids and nitrogenous bodies of a protein nature are also present.

Theoretically, the hydrolysis of any one starch is a composite of at least two reactions: the hydrolysis of linear chains of comparatively low molecular weight, and the hydrolysis of very large, branched structures. A fundamental study of the hydrolytic reaction should logically proceed from a study of each reaction separately to a study of the hydrolysis of a mixture of these two molecular species. Practically no information of this nature has been reported, since these fractions have not been available in even relatively pure form until quite recently. Most of the more recent work has proceeded on the assumption that the primary reaction of hydrolysis may be viewed as an acid-catalyzed hydrolysis of 1-4 α-glucosidic linkages and that each of these bonds in starch is equivalent to the next. Little thought has been given to the availability of these various bonds or to the effect of other physical variables which might tend to impede or accelerate their scission, such as differences which arise from the fact that these bonds are links in either a linear or branched structure. In respect to availability, it would seem probable that nearly 10% of corn starch exists in a state so completely oriented that it is solubilized with difficulty in the presence of acid at the temperatures used in commercial starch hydrolyses. This portion is predominantly linear in configuration. It may be recovered as an insoluble precipitate, along with other insoluble residues, in the early stages of the hydrolysis by stopping the reaction, cooling, and filtering. Practically no information exists as to the relative rate of hydrolysis of this fraction by acid although its hydrolysis by enzymes has been the subject of several reports. Evidence pointing to the fact that some of the glucosidic linkages in starch are more susceptible to hydrolysis than others has been presented in previous chapters. This observation may account for the more rapid hydrolysis of starch in the initial part of the reaction, and the conclusion conforms with the results reported by Haworth (3) that the "disaggregation" (initial hydrolysis) of starch involves a slightly lower activation energy than that for the normal, 1-4 α-glucosidic bond.

It is assumed, furthermore, that the small number of anomalous linkages are of minor importance in a study of the kinetics of starch hydrolysis. Some workers (4) have assumed that the 1-6 α-glucosidic bond hydrolyzes at a rate at least equivalent to that of the normal 1-4 α variety. Others (5, 6, 7) have sought to show that the former linkages are more resistant to acid than the normal linkages. More recently, Swanson and Cori (8) have found from a study of the comparative rates of hydrolysis of amylose and dextran (a glucose polymer containing substantially all 1-6 α-glucoside linkages) that the 1-4 α-linkage is ruptured more easily. Moreover, the 1-4 linkage was found to possess the same stability whether in a linear or branched starch molecule.

3. Reactions Involved When Starch Is Treated with Acid. An examination of the end products of the reaction discloses the presence of glucose as the major product. In addition, depending on the conditions imposed, there are present variable quantities of gentiobiose, the isomer of gentiobiose containing a 1-6 α-glucosidic linkage, derivatives of furfural, levulinic acid, and other degradation products which result from treating carbohydrates with acid at high temperatures. In addition to the main reaction which results in the production of homologues of maltose, maltose, and finally glucose, there are formed also by hydrolysis a series of higher sugars containing at least one 1-6 α-glucosidic bond in their structure which give rise to the isogentiobiose mentioned. In addition to the primary reaction of hydrolysis, at least two other types of reactions are in progress. First, it is evident from the work of Berlin (9) and others that the gentiobiose, which can be isolated from starch conversion liquors, can be accounted for by a rejoining of 2 glucose molecules into a dissaccharide containing a 1-6 β-glucosidic bond. Secondly, it would appear that after glucose is formed it may be degraded in the presence of acid catalysts at high temperatures to non-carbohydrate bodies. Obviously, both of these secondary reactions detract not only from the yield of dextrose, maltose, and related sugars, but also from the quality of such products in respect to taste and color, particularly when the end-product is finished as a sirup of high sugar content. When the sugar is crystallized from the conversion liquors, the mother liquor, or "hydrol" as it is referred to in dextrose manufacture, is of unpleasant taste, and its utility is restricted because of the undesirable properties of the by-products present. In consequence there is a very material decrease in the yield of sugar.

To facilitate discussion, the primary reaction may be represented by the equation

$$\underset{\text{Starch}}{(C_6H_{12}O_6)(C_6H_{10}O_5)_x} + xH_2O \rightarrow \underset{\text{Dextrose}}{(1 + x)(C_6H_{12}O_6)}$$

As the concentration of dextrose increases, the first secondary reaction is induced to proceed to the right:

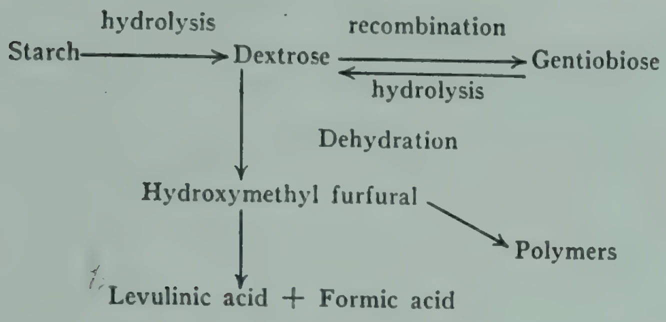
$$\underset{\text{Dextrose}}{2C_6H_{12}O_6} \rightleftharpoons \underset{\text{Gentiobiose}}{C_{12}H_{22}O_{11}} + H_2O$$

It is also quite possible that dextrose may rejoin with maltose and its homologues by means of 1-6 β-glucosidic linkages as well. This reaction is reversible (10). The reconversion of "hydrol" to increase the yield of dextrose has long been known to the industry (11). The primary reaction is obviously not reversible, since dextrose reacts in the presence of acid to form the gentiobiose type of polymer.

The secondary reaction involving the production of the furfural type of dehydration by sugars in the presence of acids, in the case of dextrose, most likely results in the formation of hydroxymethyl furfural. It is also quite possible that the yields of levulinic acid in the acid treatment of starch and dextrose, discussed by Moyer (12), may originate by way of hydroxymethyl furfural. The last two products may polymerize to substances of unknown composition. In addition to these products, others of low molecular weight may also result.

Diagrammatically, then, the action of acid on starch may be represented in its essentials by the accompanying composite scheme. (P. 379.)

Because of the complexity indicated, it would seem extremely difficult to study the process quantitatively. Within the range of conditions employed in practice, however, the vertically written reaction occurs to only a fraction of 1%. Hence it is possible, by confining the study to the limitations of commercial practice, to obtain approximate results for the two major reactions, hydrolysis and recombination, which may then be corrected for the



effect of the destructive reactions. With the knowledge that the reversion is an equilibrium reaction, its effect may be minimized in a study of acid hydrolysis by selecting conditions conducive to low concentration of dextrose; *e.g.*, dilute solutions or starch in the early stages of commercial conversions. The effect of the several variables on each component part of the reaction may then be determined.

4. **Hydrolysis Reaction.** Freudenberg, Kuhn, and others (13-15) have proceeded on the assumption that the hydrolysis is a monomolecular reaction involving the scission of equivalent, 1-4 α-glucosidic bonds. They have applied a simple statistical treatment to predict values to be obtained for glucose, maltose, and higher homologues for any given stage in the early stages of the hydrolysis. The assumption should also be made that the few 1-6 α linkages (possibly 5% of the total) are hydrolyzed, if at all, under the conditons employed in the last phases of the conversion. From the equations developed, these workers have determined the rate at which any degradation product of any given length is formed and the maximum yield of this product which may be obtained during a hydrolysis of a polysaccharide chain. According to Freudenberg and Kuhn, the maximum yield, $\phi_{\text{max.}}$, as a fraction of the starting material, is equal to the number of chains of length n, Z_n, times n units per chain, divided by N, the total number of units.

$$\phi_{\text{max.}} = \frac{nZ_{n\,\text{max.}}}{N} = n\left(\frac{2}{n+1}\right)^2\left(\frac{n-1}{n+1}\right)^{(n-1)}$$

Calculations for monose, biose, triose, and tetrose are given in Table XXVIII. The theoretical course of the reaction is shown diagrammatically in Fig. 94.

To verify these results for starch by experiment, however, requires better analytical methods than the determination of total reducing sugars by Fehling's test, polarimetric measurements, diastatic analyses, or similar approximations which have been applied in earlier work. Cantor and Moyer (16) have discussed the development of applicable analytical methods, especially those which have been reported recently. These include the method of Sichert and Bleyer (17) to estimate dextrose by use of a modified Barfoed

TABLE XXVIII

Application of Statistical Method to Starch Hydrolysis

Fragment	Maximum yield at 25% hydrolysis	Maximum yield at 50% hydrolysis	Maximum yield at 75% hydrolysis	Maximum yield	Hydrolysis at maximum yield
	per cent	per cent	per cent	per cent	per cent
Monose	6.3	25.0	56.2	100.0	100.0
Biose	9.4	25.0	28.1	29.8	66.7
Triose	10.5	18.8	10.5	18.8	50.0
Tetrose	10.5	12.5	3.2	13.8	40.0

reagent (copper sulfate and sodium acetate), the method of Hurd and Cantor (18) to estimate the lower sugars by methylation and fractional distillation, the method of Hurd, Liggett, and Gordon (19-21) by which the lower sugars are converted to propionic esters and are fractionally distilled, and a modified method of the A. O. A. C. for the determination of dextrins by alcoholic precipitation and correction for the reducing substances in the precipitate.

Cantor and Moyer have discussed the accuracy of the above methods and have compared the results of analyses of sirups representing various stages in the acid hydrolysis of corn starch. The percentage composition of conversion liquors over the range of 25 to 60% hydrolysis is shown in Fig. 95. The assumption is made that the total reducing value by Fehling's test, calculated as per cent dextrose (dextrose equivalent, D. E.), expresses the degree of hydrolysis. In Table XXIX the results of analysis of a corn starch sirup at 42 D. E. are compared with the results of statistical analyses. From a comparison of these values and from a comparison of other values given in Figs. 94 and 95, it will be seen that only in the case of maltose is there an agreement between found and calculated values. The percentage of dextrose found is decidedly higher than was anticipated, and the amounts of higher sugars are considerably lower than the predicted ones. It is obvious,

TABLE XXIX

Comparison of Statistical and Actual Analyses of Acid-Modified Corn Syrup

Constituent	Statistical analysis	Actual analysis
	per cent	per cent
Dextrose	17.7	22.1
Maltose	20.6	20.8
Maltotriose	17.9	15.3
Maltotetrose	13.3	4.5

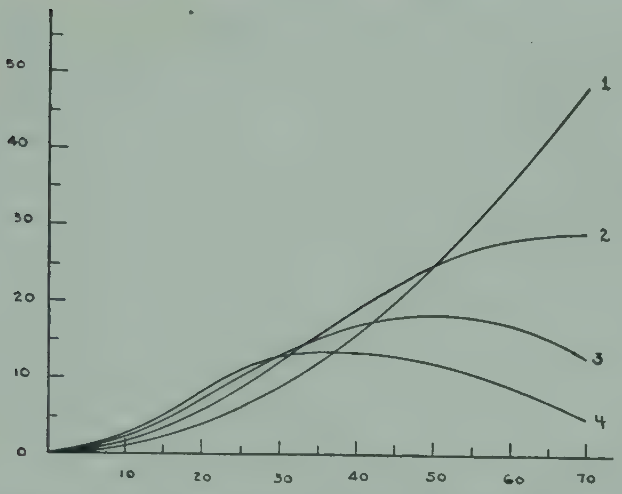


FIG. 94. Theoretical course of starch hydrolysis by statistical analysis. Curve 1, monose produced; Curve 2, biose produced; Curve 3, triose produced; Curve 4, tetrose produced. Abscissa, per cent degradation; ordinate, per cent yield.

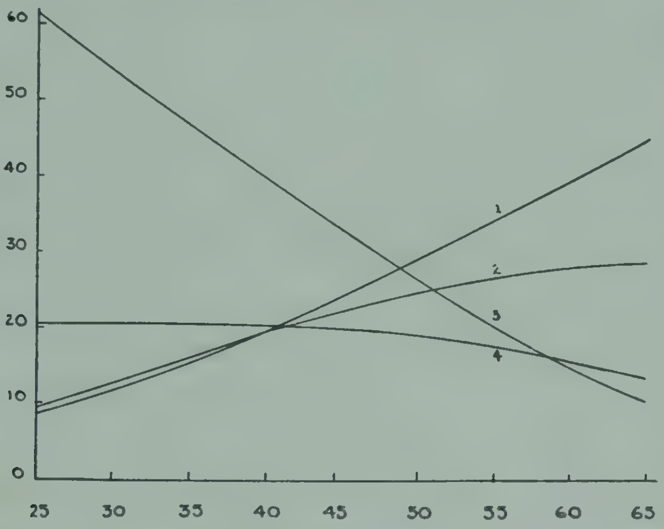


FIG. 95. Percentage composition of starch conversion liquors. Curve 1, dextrose; Curve 2, maltose; Curve 3, dextrins; Curve 4, higher sugars. Abscissa, dextrose equivalent; ordinate, per cent composition.

therefore, that the course of starch hydrolysis cannot be considered as a random hydrolysis of equivalent glucosidic linkages even in the early part of the reaction and that the suggested course of the hydrolysis is an oversimplification of the actual manner in which starch is degraded.

Inasmuch as the reaction rate for maltose is higher than the average rate for starch, Cantor and Moyer have proposed that a terminal dextrose unit is more easily hydrolyzed in order to account for the higher amount of dextrose found. This supposition would seem logical and a similar explanation has been given by Myrbäck (7). However, it would also seem probable that any maltose which is formed in the course of hydrolysis would, for this same reason, be more speedily hydrolyzed to dextrose than the rate at which more maltose is formed by random rupture of glucosidic bonds. The amount of maltose present at any given time consequently should be decidedly less than would be anticipated from calculations by the statistical method. Therefore, the agreement between the per cent of maltose found by analysis and that predicted is not significant, but rather must be fortuitous. It might also be recalled that the fact that the rate of hydrolysis of maltose is higher than the rate for starch led to the supposition which prevailed for several years in the industry that corn sirups contain very little maltose. The issue was settled by Berlin (22) who demonstrated the presence of substantial quantities of this sugar in these sirups.

Possibly some error is involved in the study of the kinetics of the reaction by using copper reduction values calculated as percentage of dextrose (D. E.) as a direct measure of the percentage of glucosidic bonds hydrolyzed in starch. In particular, the method appears to be unsuitable to detect the extent of hydrolysis where a scission is made which results in an aldehyde group attached to a long chain of glucopyranose units (a so called, non-reducing dextrin). Rather, the results of the Fehling's test give greater emphasis to the proportion of shorter chains which may be present in the hydrolysis mixture at any given time. From the work reported by Levine *et al.* (4) it would appear that every scission which results in the formation of an aldehyde group, whether the latter is a part of a short or relatively long chain, can be correctly determined by oxidation with iodine. Sadovy (23) has followed the acid hydrolysis of starch by measuring the amount of liberated aldehyde groups iodometrically.

Applying the monomolecular reaction equation to his results, Sadovy finds that the reaction constant decreases during the reaction. Taking an average of the values for K during the early part of the reaction and using the equation

$$K = \frac{2.3(2 - \log\,(100 - Z))}{Ct}$$

where Z is the hydrolysis product, expressed as per cent of total dry substance, C is the volume per cent acid, and t is the time in minutes, no difference in the average value was found for a change in the starch concentration. A value of K equal to 0.47 was found when 12% of dry substance (by volume) was used in the hydrolysis; a value for K equal to 0.48 was found when 22.3% dry substance was used. Quite obviously, had the starch concentration been increased to very high values, for example to double the concentration investigated, it would be expected that the reaction constant would have decreased owing to the effect of a recombination of the dextrose, as mentioned previously.

The temperature of hydrolysis used by Sadovy was 133°C. and an acidity of 0.1% HCl (by volume) was employed. With the same acidity, the value of K was found to vary with a change in the temperature of hydrolysis according to the equation

$$\log K = 0.0418t - 5.884$$

The determined values for K at various temperatures are compared with those calculated for K according to the above equation in Table XXX. It will be seen that each 10°C. rise in temperature results in very nearly a threefold increase in the value for K. The critical increment, E, may be calculated by means of the Arrhenius equation

TABLE XXX

Actual and Calculated Values for Starch Hydrolysis. Reaction Rate Constant at Various Temperatures of Hydrolysis

Hydrolysis temperature °C.	K by experiment	K by equation
119	0.125	0.123
133	0.470	0.473
138	0.770	0.766
143	1.200	1.230

$$2.3 \log \frac{K_2}{K_1} = \frac{E}{R} \quad \left(\frac{T_2 - T_1}{T_2 T_1} \right)$$

where K_2 and K_1 are the reaction rate constants at the absolute temperatures, T_2 and T_1, respectively and R is the gas constant, 1.98 cal. E is found to be 31,000 cal.

Within a limited range of hydrochloric acid concentration, Sadovy finds that the rate of hydrolysis is proportional to the acid added, expressed as per cent by volume. Quite naturally, however, this relationship could not be expected to hold at extreme acid concentrations. The acid-binding power of the starch, to be discussed in a following section, would materially lower the acid activity at extreme dilutions. At high acid concentrations, particularly if the less ionized acids are used, the acid activity would not be proportional to the amount of acid added.

5. Recombination. The rate of glucose recombination, or reversion as it is frequently called, has been studied by Moelwyn-Hughes (24). The reaction of 10, 20, 30, 40, and 50% dextrose solutions, in normal HCl at

60° and 70°C., was followed polarimetrically. This investigator found that the reaction proceeded with a progressive decrease in the dextrose content until an equilibrium was reached in each case. Both the position of the equilibrium and the rate of the reaction in approaching this equilibrium are independent of the initial dextrose concentration. The critical increment for the reaction is about 33,500 cal., which is substantially higher than the value for starch hydrolysis. Hence the recombination reaction increases more rapidly with rising temperature than the hydrolysis reaction. Seaman (25) also determined the energy of activation, but at higher temperatures and in more dilute acid. When the reaction was followed by noting the decrease in fermentable sugar caused by heating dextrose in 0.4% sulfuric acid, the critical increment was found to be 33,200 calories over the temperature range of 170-190°C.; when decrease in reducing power was used to follow the reaction, E was found to be 33,100 cal. The value dropped off slightly in both cases to 32,100 cal. as the acid concentration was raised to 1.6%. It may be concluded therefore, that the recombination reaction increases more rapidly with rising temperature than the hydrolysis reaction and in consequence, very high converting temperatures are to be avoided, or otherwise the yield of dextrose will suffer accordingly. Seaman also found that the reaction was of the first order.

Silin and Sapedina (10) have investigated the recombination reaction and have determined the equilibrium point for many sets of experimental conditions. From their data, they have developed a modified bimolecular equation for the reaction

$$K = \frac{An^2}{100(100-n)}$$

where K is the reaction constant, A is the initial glucose concentration in the percentage by weight of the water present, and n is the glucose remaining at equilibrium as the percentage of the initial amount. The constant, K, was found to be 259 over the range of 8.6 to 62.9% of the initial glucose concentration (or 9.4 to 170% by weight of water present). If the determined value is substituted for K and n is calculated, the percentage of dextrose remaining at equilibrium for any initial dextrose concentration is given by the equation

$$n = \frac{100(\sqrt{1 + 0.015444A} - 1)}{0.007722A}$$

Table XXXI gives a series of dextrose equilibria values. It is to be noted that at a dextrose concentration of 20% (20 g. of dextrose in 100 cc. of solution) only about 92% of the dextrose remains at equilibrium. In hydrolysis it is evident that the use of starch of high concentration results in

TABLE XXXI

Dextrose Reversion Equilibria at 100°C.

Initial concentration of starch, per cent by weight	Initial concentration, based on dextrose Per cent weight of mass	Per cent weight of water	Per cent dextrose (dry basis) at equilibrium
0.0	0.0	0.00	100.0
4.5	5	5.26	98.1
9.0	10	11.11	96.0
13.5	15	17.65	94.0
18.0	20	25.0	91.8
22.5	25	33.3	89.6
27.0	30	42.9	87.4
36.0	40	66.7	82.5
45.0	50	100.0	77.1
54.0	60	150.0	70.9
63.0	70	233.3	63.6

reduced dextrose yields; whereas the lower the starch concentration used, the higher will be the final yield of dextrose.

The practical aspects of these observations are emphasized by Silin and Sapedina. They point out that in starch conversion concentrations which should lead to a final dextrose concentration of 25% at the end of the hydrolysis, result in the production of 89.6% of this amount of dextrose. The remaining 10.4% consists principally of the products of reversion (such as gentiobiose). After crystallization of the dextrose, the "hydrol" (mother liquor) contains this 10.4%. Since the "purity" of "hydrol" in respect to dextrose is 66.7%, according to these workers twice as much dextrose is present in "hydrol" as the total products of reversion. The 10.4% of the latter prevents the crystallization of 20.8% of dextrose. Therefore, not 10.4%, but 10.4% + 20.8% = 31.2%, or nearly a third of the expected dextrose yield, is lost owing to the total effects of the recombination reaction.

Silin and Sapedina point out that instead of a reconversion of "hydrol" to recover a part of this dextrose loss, it would be more practical to resort to a lower starch concentration in the initial conversion. Reconversion requires dilution and the addition of more acid and more sodium carbonate. The added water is as expensive to evaporate after reconversion as after the primary conversion. The addition of acid and sodium carbonate materially increases the sodium chloride content of the reconverted "hydrol" and salts of this type are known to affect the crystallization of dextrose adversely. These investigators propose the use of a starch concentration which will result in a 15% solution of dextrose, if the yield was 100% of the theoretical. At this concentration, the purity of the sirup is 94% (as dextrose) at equilibrium, only 6% being lost in recombination. The "hydrol" therefore contains 6% + 12% = 18% of solids to be considered as a loss of dextrose. Assuming that a 25% concentration (as dextrose) represents normal conversion, a reduction of the concentration to 15% results in an increase in the dextrose yield of 13.2% above normal.

It should be pointed out that the 25% concentration is not commonly used in America for dextrose production. For many years starch suspensions of 12°Bé. have been used which have a solids content in the neighborhood of 18%, allowances being made for a chemical gain in water during hydrolysis and for the dilution caused by the condensation of the steam used to supply the heat for the reaction. Experiments performed by W. B. Newkirk and his associates have shown that this concentration is the optimum in respect to the economy of the process; that is, when the cost of a larger volume of water to evaporate (owing to higher dilution) and other factors are balanced against the value of the increased yield of dextrose which results from the use of a low concentration of starch.

The "hydrol," or mother liquor from dextrose crystallization, has been extensively examined in order to shed further light on the nature of the complex reaction which results when starch is hydrolyzed with acid. Miller (26) describes "hydrol" as a dark colored, viscous sirup containing 78.3% solids and 21.7% water. The solids contain 7.93% ash. Hurd and Cantor (18) report the analysis of "hydrol" in respect to sugars as 40.1% monosaccharides, 28.5% disaccharides, and 4.0% trisaccharides. The analysis was performed by the methylation and fractional distillation of the component sugars. Miller (26) succeeded in obtaining the acetate of gentiobiose directly from "hydrol," whereas Berlin first rid the "hydrol" of fermentable sugars by the action of yeast. Among the nonfermentable sugars, Miller found a substantial quantity of another disaccharide in addition to gentiobiose. He has prepared derivatives of this sugar and has described their properties. It may be the same sugar as the isomer of gentiobiose found in "hydrol" by Coleman *et al.* (27). The presence of 6-(α-glucosyl)-glucose in "hydrol" could, moreover, be accounted for as an incompletely hydrolyzed residue from the amylopectin fraction of starch.

6. Intramolecular Dehydration and Other Destructive Reactions. The destructive or irreversible reactions into which dextrose enters in the presence of acid have received very little quantitative study. The actual loss in yield which results from the formation of products of the furfural and levulinic acid class is relatively small under the conditions normally used in sirup and dextrose manufacture. Means of measuring with accuracy very small concentrations of these products have been lacking. The undesirability of these side reactions in practice is due more to their reflection on the quality than on the quantity of the primary products.

The production of levulinic acid is slight during the time allowed for conversion of the starch to sirup or sugar. This is evident from the data submitted in recent patents issued to Thompson (28) and to Moyer (29) for the production of this acid from starch or starch products. Thompson suggests treating 200 g. of carbohydrate in 400 cc. of water with enough HCl to give a concentration of 6% hydrochloric acid and heating at the boiling point of water, under a reflux, for 22 hours. The hydrochloric acid, together with the formic acid which is formed simultaneously with the levulinic acid, is distilled off, and the levulinic acid is recovered after a vacuum distillation. The use of "hydrol" is recommended as a substrate for this destructive reaction. Not only would "hydrol" seem a logical choice for reasons of economy, but also because it might be concluded that it already contains some levulinic acid as a result of the primary acid treatment of the starch from which the "hydrol" is produced. Thompson also found "hydrol" suitable for use in that it contains electrolytes which increase the acid activity of the added HCl. From 265 g. of "hydrol," 48 g. of levulinic acid were recovered. The yield of levulinic acid was found to vary inversely with the concentration of "hydrol" in the solution treated. Yields of levulinic acid as high as 35%, based on the total dry substance, are reported when the "hydrol" concentration was reduced to 19 g. in 400 cc. of water.

Moyer suggests a treatment in which starch and HCl are preheated to 100°C. at atmospheric pressure in an initial step. The strength of HCl used is between 0.5 and 3.5%. Starch is used in such a concentration that a molar ratio of hydrogen chloride to dextrose units is maintained at 0.15 to 0.25. Suspensions of the starch of 18.5°Bé are recommended for the process. After the preliminary digestion, the liquors are transferred to a pressure cooker where the temperature of the reaction is slowly raised to between 175° and 215°C., after which the reaction mixture is held at 190-200°C. until the desired results are obtained. A controlled method, such as by the introduction of live steam, is suggested for raising the temperature from 100° to 200°C. so that the rate of heating may be held to a temperature rise of about 2°C. per minute. Faster heating unduly extends the dextrose destruction beyond the levulinic acid stage to the point where polymerization, leading to the formation of humin, is excessive. The production of non-condensable gases also attends humin formation.

The disappearance of dextrose is followed polarimetrically, and the time is noted when 99% of the substrate has reacted. Levulinic acid does not immediately appear in proportion to the amount of dextrose which has reacted. This result substantiates the conclusion that the acid forms through an intermediate reaction, quite possibly the formation of hydroxymethyl furfural. Therefore, the heating period is extended twice as long as the time required for 99% of the initial dextrose to disappear from the solution. Moyer treats 162 lbs. of starch suspended in water at a density of 18.5°Bé. by adding 32 lbs. of 28% HCl and raising the temperature from 100° to 200°C. over a period of 50 min. It requires about 25 minutes at 200°C. to produce a yield of 56 lbs. of levulinic acid. This is 48.5% of the theoretical yield. The total reaction time is 50 plus 25 which equals 75 minutes. Formic acid, produced simultaneously with the levulinic acid, is removed by distillation after partial neutralization of the liquors to pH 1.9 with sodium carbonate.

If we assume that levulinic and formic acids originate from hydroxymethyl furfural, it would seem reasonable to suppose that both form in equimolar proportions.* According to these assumptions, the amount of each produced in the acid hydrolysis of starch may be estimated from the difference between the total acid present after conversion, determined by potentiometric titration, and the amount of hydrochloric acid added.

Techniques to measure quantitatively the amounts of the precursor of these acids at different points in the acid hydrolysis of starch have been lacking. An advance was made in this phase of the study, therefore, by the observation of Cantor and Peniston (31) that hydroxymethyl furfural may be determined by the use of the polarograph. These workers found that this substance is reducible at the dropping mercury electrode. The half wave potential is sufficiently removed from that observed for dextrose and maltose by Kerr (32) that measurements of the wave height offer a good method for quantitative estimation in starch hydrolyzates. Hydroxymethyl furfural has been shown to be present in "hydrol" by Cantor and Molteni [quoted by Miller (26)] by this method.

The extent of the destructive reactions may now be determined by taking the sum of the amount of hydroxymethyl furfural found by the polarographic method and the amounts of levulinic and formic acids estimated by titration.

No published data on the rate of hydroxymethyl furfural formation are as yet available. However, from private communications with the originators of the polarographic technique to measure this quantity it is known that the critical increment in the temperature range of 120-142°C. is quite close to that for the recombination of dextrose. Hence the rate of hydroxymethyl furfural formation, as is also the case for the recombination reaction, is increased more in proportion than the hydrolysis reaction as the temperature is raised. The rate of the destructive reaction is proportional to the effective acid concentration.

---

*The work of Teunissen (30) justifies this assumption.

Recently, Singh *et al.* (33) have studied the degradation of glucose to, and the production of colored bodies from hydroxymethyl furfural by means of its absorption spectra in the ultraviolet. From the changes in relative optical densities at the two characteristic peaks, 230 mμ and 284 mμ, as the reaction proceeds, they were led to believe that the first reaction consists of an opening of the pyranose ring in glucose, development of a carbonyl group, followed by dehydration. Later, color develops in the solution for which hydroxymethyl furfural is the precursor. Levulinic acid solutions showed no apparent destruction on heating. Simultaneously, from similar studies involving measurement of light absorption in the ultraviolet, Pacsu and Hiller (34) proposed a mechanism consisting of opening of the glucose ring followed by the formation of a molecular compound between acid molecules and the open-chain form of the sugar, and Wolfrom *et al.* (35) have proposed an opening of the pyranose ring, the creation of a double bond between carbons 1 and 2, followed by ring closure from carbons 2 and 5, and further dehydration to create the furan structure.

Sadovy (23) has estimated the combined effects of the recombination reaction and of the destructive reactions by a determination of the loss in dextrose which results when pure dextrose solutions are heated with acid under fixed conditions. Twenty per cent solutions to which 0.1% of HCl (by volume) was added were heated at 143°C. for various periods of time. When starch is treated with acid, under comparable conditions, it is found that the amount of dextrose present during the latter stages of the reaction is less than is estimated by applying a correction for dextrose reversion and destruction obtained according to the manner indicated above. For example, after a heating period of 45 minutes, Sadovy found that dextrose solutions have decreased only 3% in their dextrose content. After a comparable treatment of starch, the dextrose content of the reaction mixture indicates that about 8% of the dextrose produced has either recombined or has been destroyed. Sadovy explained the discrepancy by assuming that a part of the starch does not undergo hydrolysis. At least it would seem plausible to conclude that many of the glucosidic bonds in starch do not hydrolyze at the rate anticipated.

In summation, therefore, it may be stated that even though corrections are made for the effects of secondary reactions, the reaction rate for the hydrolysis of starch is greater in the initial stages, then equals, and finally becomes less than that calculated by means of a monomolecular reaction equation for a strictly random hydrolysis of equivalent linkages. The discrepancies will be explained, no doubt, when the problem is approached with due regard for the physical chemistry involved, as for example by recognizing the several possibilities: (*a*) that the hydrolysis observed is a composite of at least two reactions, the splitting of comparatively short linear chains of glucopyranose units and the splitting of much larger branched structures; (*b*) that some glucosidic bonds are preferentially hydrolyzed owing to physical forces which place them under strain; (*c*) that not all of the glucosidic linkages are equally available to the activity of the acid owing to the ability of certain component parts of the starch to orient into resistant complexes; and (*d*) that there are types of glucosidic linkages present in starch components which no doubt require a higher activation energy than that for the normal or predominating type. The more recent advances in starch chemistry, described in previous chapters on the physical and chemical properties of the starches, should make the problem of starch hydrolysis approachable by a logical plan of investigation, and the kinetics of this complex reaction should shortly become known.

7. Effect of the Substrate Starch on the Acid Concentration. The effect of the starch on the concentration of the active acid during hydrolysis has been the subject of many researches. It has been known for many years that the acid activity in hydrolysis mixtures containing starch is not proportional to the amount of added acid but that it is usually a value less than this amount. Attempts have been made to explain this discrepancy by assuming that the starch, some part of it, or some impurity associated with the starch either combines with the acid or acts as a buffer. Since the rate of hydrolysis is proportional to the acid activity, the study of methods to purify or prepare the starch for hydrolysis assumes importance because variable acidities lead to variable results and possibly to losses in yield. Such treatment of starch might be one of additional refining or purification from interfering impurities or, when this is not possible, of the production of an "acid starch" with a buffer capacity much less than that of the untreated starch.

One of the earlier investigators of the problem, Silin (36) found that as much as 40% of the sulfuric acid added to hydrolyze potato starch may be bound by this substrate. Widmeyer and Rall (37) studied the effect and expressing their results on a basis of the percentage of starch used, found that corn starch binds between 0.022 and 0.087% of HCl, while potato starch binds 0.052%. Smirnov (38) has recently reviewed the subject. He has pointed out the acid-binding capacity of impurities and other residues associated with the starch. Among those considered are proteins, fats, cellulose-like material, SiO_2, phosphates, and other salts of Ca, Mg, K and Na. Of these, nitrogenous and phosphoric acid residues appear to exert the greatest buffering effect, particularly the type of protein which might be associated with potato starch. However, no constant figure is arrived at for the acid-binding power of starch, either of corn or potato, since the composition of each type varies with the different samples tested. Of twelve samples analyzed and inspected for acid-binding power, however, it was found that this power could be correlated with starch composition in respect to the two residues mentioned. Although the basic constituents of the ash might be expected to exert a certain amount of acid-binding, it is pointed out that the composition varies widely in respect to these constituents. This difference in ash content is to be expected inasmuch as starch is a fairly effective adsorbent and inasmuch as different mills show considerable variations in the compositions of their various mill liquors. This is particularly true in respect to the cations present. For example, starch-producing mills which are also engaged in glucose production frequently use either softened water or steam condensate or a combination of the two as a source of water for their starch-milling and converting processes. The natural Mg, K, and Ca ions associated with native starch are therefore being continuously removed throughout the milling process or being continuously replaced with sodium ions as the starch comes in contact with liquors progressively higher in sodium ion content. Samec (39) has adequately discussed the adsorptive capacity of starch in respect to metallic ions and ammonia. The practical result of the replacement of the natural cations of starch by sodium ions is that starch sirups made from the hydrolysis liquors of such starch show decidedly less tendency to become turbid as the result of the formation of insoluble sulfates. (The sulfate ion arises from the sulfite used for the bacteriological control of the wet milling processes.) The nitrogenous material adsorbed on starch, some types of which appear to accelerate the formation of color during and after hydrolysis, likewise varies from starch to starch, particularly in respect to the class of nitrogenous substance. This difference also results, in part, from variations in the composition of process waters employed in milling, owing to fluctuations in the control of their biological activity.

In the case of some starches, potato, for example, most of the phosphate found with the starch is esterified with constituents of branched configuration, the amylopectins. Samec (39) has shown that this acid phosphate group is liberated in the very early stages of hydrolysis but even though it were not, the amylopectin acid phosphate should exert a measurable amount of buffering action. The chemically bound phosphate in corn starch is, no doubt, too insignificant in amount to account for or to be considered in calculations of the acid-binding power of the starch. The buffer in this case is undoubtedly principally nitrogenous in character. In corn starch mills producing sirups and dextrose, the remainder of the acid-binding power of the starch may be attributed to the sodium salts of weak acids, such as phosphoric and lactic, which have been adsorbed by the starch during milling. Acid phosphate arises from the hydrolysis of phytin, the phosphorus reserve of the corn kernel.

Because of the wide variation found in respect to starch composition, Smirnov recommends that the acid-binding power of starch used in hydrolysis be determined periodically. Two methods are suggested. The first method was originated by Silin (36) who calculated the amount of bound acid from the deviation in the anticipated proportionate increase in the rate of hydrolysis compared with the increase in acid concentration. The method is as follows: 30 g. of starch (dry basis) and 50 cc. of water are preheated to 55°C. for 15 minutes. Based on the weight of starch to be treated, 0.182% of N HCl is prepared in 100 cc. of water. The acid is boiled, and the preheated starch suspension is added gradually over a period of 5 minutes. The mixture is made up to 250 cc. and then boiled under a reflux. The hydrolysis is followed by an iodometric analysis for reducing groups. A duplicate hydrolysis is carried out with the same starch with 0.364% of acid. Assuming that the starch contains impurities which bind x% of acid, the time required to reach a certain limiting value in the hydrolysis for the lesser amount of acid, T_1, and for the larger amount of acid, T_2, being known, then

$$\frac{0.364-x}{0.182-x}=\frac{T_1}{T_2}$$

Solving for x gives the amount of acid bound as per cent of dry starch.

It would seem that this method or an elaboration of it possesses particular merit for several reasons. First, not only is the effect of starch residues which reduce the acid activity determined, but the result is corrected for the effect of increased acid activity exerted, possibly, either by the carbohydrate itself or substances associated with the starch. Secondly, the determination is made, or can be made, at the elevated temperature used in hydrolysis, and hence the influence of the buffers is determined on the actual acid activity at this elevated temperature. Both of these, the buffering effect and the acid activity before buffering, are probably different at lower temperatures from what they are at higher temperatures.

The effect of electrolytes, in particular NaCl, in increasing the acid activity when carbohydrates are treated with acid has been noted by several workers including Thompson (28). The amount of such electrolytes as NaCl present in the starch is therefore important in practice for the prediction of the rate of hydrolysis, for a given addition of acid. The method of Silin for estimating the effect of the substrate on the acid activity of a given addition of acid should take into account and correct for the possible "salt effect" of the starch used.

The second method used by Smirnov for estimating the acid-binding capacity of starch was apparently suggested by Widmeyer and Rall (37). The hydrolysis mixture of starch and acid is prepared as in the first method. It is then autoclaved for 7 to 8 minutes, cooled to room temperature, and enough water is added to make the total weight 450 g. The solution is titrated potentiometrically with 0.1 N NaOH. The type of titration curve obtained is shown in Fig. 96. An inflection may be noted in the neighborhood of pH 4.5. Hence, the difference in the amount of acid added to the starch and the amount corresponding to the alkali used to titrate the hydrolyzate to pH 4.5 is taken as the amount of acid bound by the starch.

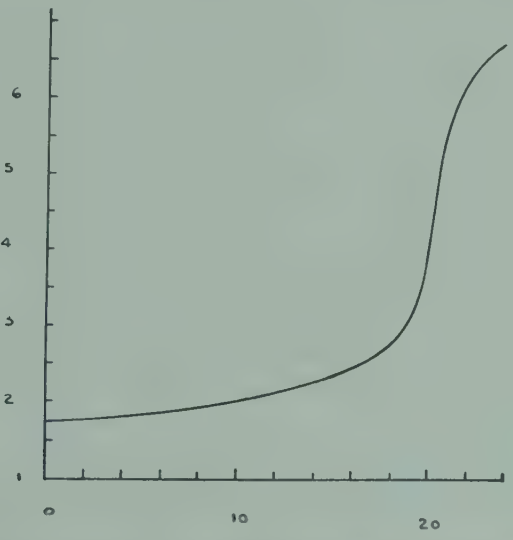


FIG. 96. Potentiometric titration of hydrolyzed starch. Abscissa, cc. 0.1 N NaOH; ordinate, pH.

Although the use of these methods for the determination of the effect of the substrate on the acid activity affords a means to estimate the true acid activity in starch hydrolysis, their use in practice has certain limitations. Not only will the acid-binding power of starch be found to vary with the type of starch, *i.e.* corn and potato, and with the starch-milling process employed, but even within a mill the starch made will be found to vary from time to time with different batches of starch. This requires that each batch of starch be tested and the results determined before it is hydrolyzed. It would seem more logical to develop better means for pretreating the starch in manufacture to secure a purification from or neutralization of interfering constituents as far as possible.

8. Neutralization and Clarification. In the manufacture of sirups and sugars from the hydrolysis of starch, neutralization of the acid used is commonly accomplished by the addition of sodium carbonate. Following this step, the liquors are clarified by filtration to remove suspensoids and other colloidal material including certain colored bodies. In earlier processes the liquors were allowed to settle, they were passed over vacuum filters, sometimes precoated or they were submitted to both procedures. Bone char filters were and are used for final clarification. Apparently the two steps of the manufacturing process, neutralization and clarification, are intimately connected in some respects.

Liquors from an extended acid hydrolysis of corn starch, such as those employed for dextrose manufacture, contain as insoluble or readily precipitable material small amounts of fatty acids, protein bodies, and lesser amounts of fiber and inorganic constituents. A less extensive hydrolysis, such as is used in the production of various sirups, results also in the presence of small amounts of carbohydrate material, sometimes referred to as insoluble dextrins. Quite likely this material represents the unconverted residues from a part of the amylose fraction of the starch. Paine and Badollet (40) observed that the flocculation of colloids after starch hydrolysis is influenced by the pH to which the liquors are neutralized. They suggested the use of pH control in the neutralization in order to arrive at what appears to be an isoelectric point for the coagulum and hence to obtain the precipitation of a maximal amount of this material. They have also noted that the character of the precipitate obtained in a sugar conversion varies in some respects from that obtained from a conversion to produce a sirup. For example, the isoelectric point is slightly different in the two cases, being about pH 5.0 for the former and 4.5 for the latter. In another publication Badollet and Paine (41) emphasized the point that, if sodium carbonate is added to starch hydrolyzates in excess of the amount necessary to adjust the pH to the isoelectric point, a part at least of the suspensoids redissolves, thus placing an undue load on the bone char filters. Inasmuch as the colloidal material present possesses a positive charge, these workers have proposed the use of such adsorbents as aluminates, aluminum silicate, and, particularly, bentonite, which have a negative charge at the pH employed for the clarification of starch hydrolyzates. The bentonite may be used for clarification directly after the acid conversion. Its use apparently possesses the advantage over the use of sodium carbonate in that a slight excess of the clay does not redisperse the colloidal impurities in the liquor. Badollet and Paine reported the satisfactory use in practice of sodium aluminate for clarification, the aluminate being added in such amounts as to bring the pH of the converted liquors to the isoelectric point.

The uncontrolled use of sodium carbonate for neutralization possesses another distinct disadvantage in that glucose solutions develop color at an accelerated rate if the pH is raised to 5 or above, particularly when they are treated at elevated temperatures, as has been pointed out by Sjostrom (42). This effect has been studied in further detail by Fellers *et al.* (43) and by Kroner and Kothe (44). The latter find that a sharp minimum in the formation of color occurs at pH 2.3 to 3.0 when glucose solutions are heated, a result confirmed by Singh *et al.* (33). Furthermore, Wolfrom, and coworkers (45) have studied the interaction between glucose and amino compounds of the type conceivably present in starch hydrolyzates and have found that a free amino group only is required for a decomposition reaction with enolized glucose into colored bodies at a slightly alkaline pH level of 7.5. Naturally, additional color which arises through improper neutralization technique, regardless of its source, increases the burden imposed on the bone char filters or, stated otherwise, requires an excessive ratio of a weight of bone char to clarify a given amount of sugar solution. Moreover, the efficiency of the bone char used in refining is related to the pH of the sugar solution; most chars appear to be very efficient in the pH range in which the formation of additional color is accelerated. Lowy (46) has discussed the effect of changes in pH as it modifies the decoloration of sirups by carbon and has offered an explanation for liquors that contain anions and cations on the basis that the coloring matter is electronegative and is coadsorbed with cations, particularly bivalent ones.

Deitz (47) has also discussed the effect of pH as well as other factors on the color adsorption efficiency of bone char. Dietz has measured the surface area per unit weight of bone chars and has found that it decreases often as much as 70% by usual regeneration treatments of spent bone char for reuse. Although surface area was found to be an important factor in decolorization power, it is, of course, not the only one. It was pointed out that colloidal, or suspended matter, adsorbed oxygen and other factors play important roles also.

An additional refining problem which develops from the use of acid in the hydrolysis and subsequent neutralization is that the salt so formed may adversely affect the quality and yield of the product. The early use of lime for neutralization of sulfuric acid led to the formation of gypsum which, if not removed from solution below a value represented by the solubility product constant of the ions, resulted in sirups which developed a haze of gypsum on standing. The more recent use of sodium carbonate to neutralize hydrochloric acid leads to the formation of sodium chloride. The presence of this salt, in amounts equivalent to that in liquors from dextrose crystallization has been pointed out to affect the crystallization of the sugar adversely (10, 48). The salt exerts a solubilizing effect on the sugar.

The problems outlined have instigated an intensive search for more suitable carbons and other types of adsorbents for use in sugar refining, for better methods to utilize these adjuncts, and a review of the problem of the neutralization of glucose solutions. In an early patent Duryea (49) outlined a process for treating starch hydrolyzates with 1 part of tannin to every 4800 parts of liquor, heating to 90°C., and filtering. More recently, Jurgensen and MacAllister (50) have proposed the addition of tannin to combine with nitrogenous materials, phosphoric acid to combine with metals, adjustment of the pH to about 7 with calcium or magnesium hydroxides to facilitate all reactions and the addition of an insoluble amylaceous material to act as adsorbent. Meisel (51) suggested the use of zeolites for clarification of starch hydrolyzates, claiming the advantage that all metals below sodium in the electromotive series (such as calcium and iron) are removed. A more recent patent application by Pfeiffer and Langen (52) recommended the use of zeoliths to clarify sugar liquors, and pointed out that all cations may be replaced with the hydrogen ion. Smitt (53) also proposed the use of cation exchange on glucose liquors by the use of carbonaceous zeolites. These are prepared from sawdust by carbonizing with sulfuric acid. This worker suggested a preliminary neutralization and filtration before exchange of the cations. The carbonaceous zeolites may, however, be added directly to the converter. An increased efficiency for color removal as compared to the usual bone char is noted for this specially prepared carbon. Both the ion exchange and the superior color-removing properties of this material from sawdust are attributed to a special physical structure in the adsorbent which does not develop in the carbons previously used. Walsh (54) has claimed the use of resins for the removal of acid from hydrolyzates.

Adams and Holmes (55) proposed use of a cation exchange on glucose liquors followed by an anion exchange. A resin prepared by treating tannins with formaldehyde is used for cation exchange, and a resin prepared by condensing formaldehyde with aromatic amines (56) removes the acid. The possibility of preparing sugar solutions free from both cations and anions, leaving the liquors substantially electrolyte-free, has been pointed out in detail by Liebknecht (48, 57, 58). Carbonaceous zeolites are prepared to remove cations and keratiniferous material is used to remove the anions The regeneration of the ion exchanger, so that it may be used in successive

47. Deitz, V. R., *Intern. Sugar J.*, **47**, 99 (1945).
48. Liebknecht, O., U. S. Patent 2,155,318 (1939).
49. Duryea, C. B., British Patent 280,680 (1927).
Jurgensen, R. P., and MacAllister, R. V., U. S. Patent 2,337,852 (1943).
Meisel, H., U. S. Patent 2,194,315 (1940).
Pfeiffer & Langen, German Patent App. P77,485 (1938).
Smitt, P., U. S. Patent 2,221,683 (1940).
Walsh, J. F., U. S. Patent 2,319,648 (1943).
Adams, B. A., and Holmes, E. L., U. S. Patent 2,104,501 (1939).
Adams, B. A., and Holmes, E. L., U. S. Patent 2,151,883 (1939).
57. Liebknecht, O., U. S. Patent 2,164,156 (1939).
58. Liebknecht, O., U. S. Patent 2,226,134 (1940).
59. Boyd, R. N., U. S. Patent 2,191,385 (1940).
Behrman, A. S., U. S. Patent 2,388,222 (1945).
Swain, R. C., U. S. Patent 2,251,234 (1941).
Cantor, S. M., U. S. Patent 2,328,191 (1943).
Bauman, W. C., *Ind. Eng. Chem.*, **38**, 46 (1946).
Handelman, M., and Rogge, R. H., *Chem. Eng. Progress* **44**, 583 (1948).
Walsh, J. F., U. S. Patent 2,319,649 (1943).
Krizkovsky, F. L. P., Dutch Patent 58,049 (1946).
Langlois, D. P., U. S. Patent 2,169,051 (1939).
Langlois, D. P., and Moyer, W. W., Catalytic Hydrolysis of Starch. Paper presented at the meeting of the American Chemical Society, Milwaukee, Abstracts (1938).
Bartling, F. W., *American Miller*, **75**, No. 11, 24; No. 12, 46 (1947); **76**, No. 1, 96; No. 3, 28; and No. 8, 39 (1948).
Horesi, A. C., U. S. Patent 2,359,763 (1944).
Sipyaguin, A. S., and Shoemaker, S. O., U. S. Patent 2,337,668 (1943).
Dloughy, J. E., and Kott, A., *Chem. Inds.*, **61**, 979 (1947); *Chem. Eng. Progress*, **44**, 399 (1948).
Brown, J. M., Bemis, W. A., and Monroe, F. A., U. S. Patent 2,438,033 (1948). See also, Anon. *Sugar*, p. 35 (Sept. 1944).
lendenning, K. A., and Wright, D. E., *Can. J. Research*, **F26**, 284 (1948). See also, Anon., *Food. Inds.*, **16**, 648 (1944).
Hayek, M., and Shriner, R. L., *Ind. Eng. Chem.*, **36**, 1001 (1944).
Kerr, R. W., Meisel, H., and Schink, N. F., *Ind. Eng. Chem.*, **34**, 1232 (1942).
Dale, J. K., and Langlois, D. P., U. S. Patent 2,201,609 (1940).
Monti, R. N., U. S. Patent 2,383,914 (1945).
79. Brock, F. H., and Smith, C. E., U. S. Patent 2,410,264 (1946).
Peckham, G. T., Jr., U. S. Patent 2,395,907 (1946).
Staley, A. E., Mfg. Co., and Stevens, A. H., British Patent 559,492 (1942).
Schopmeyer, H. H., Hammerstein, F. L., and Larmouth, G. W., U. S. Patent 2,429,964 (1947).
Fetzer, W. R., U. S. Patent 2,439,384 (1948).

1. Newkirk, W. B., U. S. Patent 1,471,347 (1923).
2. ewkirk, W. B., *Ind. Eng. Chem.*, **16**, 1173 (1924).
3. ewkirk, W. B., *Ind. Eng. Chem.*, **28**, 760 (1936).
4. ewkirk, W. B., *Ind. Eng. Chem.*, **31**, 18 (1939).
5. halley, M. E., *Nat'l Research Council Can., Ann. Repts.* 1138 (1943).
6. uce, T. A., U. S. Patent 2,058,852 (1936).
ntor, S. M., and Hobbs, K. C., U. S. Patent 2,354,664 (1944).

CHAPTER XV

THE AMYLASES. PROPERTIES AND PRODUCTION.

BY ERIC KNEEN

1. Introduction and Historical. Enzymes may be defined as biocatalysts; that is, as organic agents found in living matter which have specific powers for accelerating or even initiating reactions without themselves being necessarily altered in consequence thereof. In modern nomenclature the name applied to a specific enzyme is designed in such a fashion that it will give some indication either of the substrate or the reaction for which the enzyme is specific. To this is appended the suffix "ase" to indicate an enzyme. Accordingly, enzymes which degrade starch or "amylum" are designated as amylases. This should be kept in mind and caution exercised that all enzyme reactions which result in the reduction of sugar polymers to simple sugars not be labeled amylolytic. By definition, if for no other reason, the term "amylolysis" should be reserved for those reactions in which starch is involved.

The amylase group of enzymes overshadows all others in industrial significance and utilization. This has led to extensive research since the discovery of "diastase" in about 1815. In the present treatment no attempt will be made to review extensively the almost innumerable publications. Such a review would constitute a sizable volume in itself. The reader will find a fairly adequate abstract bibliography of the literature previous to 1925 in Walton's text on starch chemistry (1). A recent general review is that by Caldwell and Adams (2). Those interested in cereal processing, and particularly milling and baking technology will find of value the recent reviews by Kneen and Sandstedt (3) and Geddes (4). For the present purpose then it will suffice to give briefly the writer's concept of our present knowledge relating to the properties of those amylases of some industrial significance.

The industrial use of amylases appears to be as ancient as civilization itself. This use coincides in the beginning with the discovery that an intoxicating beverage resulted from the "fermentation" of cereal grains. It has been stated (5) that the earliest known records of the preparation of malt date back to 7000 B.C. and that beer brewing was an established craft by 5000 B.C. In the early history it may be assumed that knowledge of the mechanism of the starch-sugar transformation was very meager. The real foundation for the scientific approach to the problem of enzymatic starch degradation lies in the discovery of "diastase" by Kirchhoff (6) in about 1815 and the fine piece of research conducted by Payen and Persoz (7) in 1833. These latter authors named the active principle of barley malt "diastase," used the iodine-staining property of starch to follow its action, and even effected a degree of concentration and purification by alcohol precipitation.

Coinciding with the utilization of cereal amylases was an equally significant application of the amylases produced by the growth of microorganisms. It is evident that the alcoholic beverages produced in certain parts of the Orient were and are in large measure dependent on the action of this type of amylase. Certain types of bacteria and molds, either as cultures or as contaminants, contributed starch-degrading as well as fermentative enzymes. Mold preparations of high amylolytic activity were used in Japan for centuries, but investigations of the nature of the action and the enzymes involved did not materialize until some time after the discovery and descriptions of cereal "diastase." Possibly the first study reported in English was that of Atkinson (8) in his treatise on the diastase of "Koji." His description of the action of this fungal preparation on starch and starch degradation products was rather complete. In addition he noted the now well known property of fungal amylases; *i.e.*, their sensitivity to heat.

The wide-spread use in the Occident of malt for diastasis stimulated much research on malt amylase, particularly that of barley malt. The "component" concept, the characterization of malt diastase as a mixture of α- and β-amylase, had its origin in the postulation by Märker (9) in 1879 that there are two "diastatic ferments" in malt. Although this postulate has since developed to be an accepted fact and has even progressed so far that many believe that all amylases from any source are mixtures of α- and β-amylase, this expansion of the component concept is entirely unwarranted and not supported by the available experimental evidence. The past half century has witnessed many investigations of amylases from a wide diversity of sources. While present knowledge is by no means complete, much is known of the mode of action and the properties of many of them.

Hand in hand with the development of a more complete knowledge of the amylases, a great expansion of their use has occurred. Far from their confinement to the saccharification of starchy mashes essentially for alcoholic fermentation is the present wide-spread use of the amylases in many diverse industries. Representative uses which may be cited are the preparation of sizings, desizing, preparation of special dextrins and sugars, and the clarification of sirups and other solutions containing undesirable starch and dextrins. These applications are treated adequately in another section of this book. It becomes apparent that amylolytic starch conversion is of pronounced industrial importance. It may be anticipated that this group of enzymes will assume ever more significance in the future.

However, evidence is becoming increasingly strong (10-15) that certain of these actions, under certain circumstances and with certain enzyme systems, may be due to enzymes substantially different from the commonly recognized amylases.

2. **Classification of Amylases.** Two logical schemes of classification are apparent. One of these would involve a division based on the type of starch degradation achieved, the other a division based on the source of the amylase. In the former, starch degradation is considered to be of three main types: liquefaction, dextrinization, and saccharification. It is questionable whether any one of these can occur to the exclusion of the others. However, one of the three may be the principal action, occurring in a much more striking manner than either of the others. This type of classification is the most useful for industrial purposes and when used in conjunction with other descriptive terminology is of great value. Corollary phrases may relate to such matters as thermostability, acid stability, pH optimum, etc. Assuming the availability of adequately described amylase preparations, a competent operator should have little difficulty in selecting the one which will most satisfactorily perform the function desired under any particular plant conditions.

The classification based on the source of the enzyme obviously is of more value to the amylase producer than to the user. Also, the characteristics of the amylases in any one group tend to be similar, and this can be used as an aid in arriving at an enzyme product of desired properties. For example, if an amylase is desired that will liquefy starch at relatively high temperatures, greatest success would be anticipated by utilizing certain bacteria for production. The usual classification according to source consists of a division into three main groups: higher plants, microbes, and animals. The higher plants may be subdivided to indicate the particular plant, and again divided, especially with the cereals, to indicate whether or not the grain was germinated or malted. Amylases derived from the malts may be further divided into α- and β-amylase components. Thus, while "barley amylase" is a rather specific term, "barley malt amylase" indicates a mixture of amylases which have been resolved into barley malt α-amylase and barley malt β-amylase. Similarly, microbial amylases can be divided into groups, for example, fungal and bacterial amylases, and each division subdivided to indicate the specific organism used. Thus we have amylase from *Aspergillus oryzae,* from *Bacillus mesentericus,* etc.

It does not seem advisable to attempt a complete and detailed classification of the amylases. At best it would be cumbersome and present knowledge is not adequate to make it complete. It will suffice to keep in mind that three actions are important, starch liquefaction, dextrinization, and saccharification, and that the amylases of value in industry are derived from higher plants, particularly from cereal malts, from the growth of fungi and bacteria, and from certain glands of animals. The following sections of this chapter will outline the properties of the amylases, and the chapter by G. M. Severson will deal with the application or "use" phase.

3. Types of Activity and Evaluation.

a. General Consideration. The mode of action of an amylase essentially is that of an enzyme and more specifically of a hydrolytic enzyme. Since the kinetics of enzyme action have been discussed extensively (16-19), it is not necessary to treat in detail the concepts evolved. Van Slyke (20) in particular has presented an excellent treatise on the kinetics of hydrolytic enzymes and their relationship to the measurement of enzyme activity. In general the kinetics may be described as follows: in the initial stage of action, when the substrate concentration is great relative to that of the enzyme, the enzyme can act at full speed. The result is that under these conditions the rate of substrate degradation is proportional to the enzyme concentration and independent of that of the substrate; *i.e.*, the reaction curve is not "monomolecular" in character. In the later stages, when the substrate quantity becomes low relative to that of the enzyme, the pattern becomes that typical of monomolecular reactions. In the first stage there is a negligible time interval between combination of 1 molecule of substrate with the enzyme, its resulting hydrolysis and the release of the end-products, and the filling of the vacant space by another substrate molecule. In the second stage this time interval becomes appreciable, and the filling of vacant spaces on the enzyme is dependent on available substrate. The concept of the kinetics of hydrolytic enzymes fits some of the known facts regarding the actions of amylases on common starches (those staining blue with iodine). For example, in the saccharification of gelatinized starch by barley malt the amount of sugar produced in a given time is proportional to amylase concentration so long as the relative substrate concentration is high. However, after about 40% of the starch has been converted to sugar, the curve starts to break away from the linear relationship.

The above concept of enzyme kinetics is of prime significance in the measurement of amylase activity. As Van Slyke (20) points out, there are three possibilities for the designing of methods: (*a*) measurement of activity in the presence of an excess of substrate in order to observe conditions under which the reaction rate is proportional to the enzyme concentration, (*b*) measurement with substrate so dilute that the conversion simulates that of a monomolecular reaction, and (*c*) measurement based on the fact that, "with a given substrate solution, the time required to decompose a given fraction, say half, will vary inversely as the amount of enzyme present." All of these methods have been used in evaluating amylase activity. The various applications will be discussed under the specific types of action.

From the standpoint either of the producer or of the user, methods for evaluating amylase preparations are necessary adjuncts to successful practice. The principles for the present quantitative methods for evaluating the *saccharifying power* of malt were laid down by Kjeldahl (21) in 1879 with his "law of proportionality." He showed that up to about 40% starch conversion the rate of sugar production was proportional to the concentration of amylase. This discovery formed the basis for the later work of Lintner (22) whose "degrees Lintner" and "soluble starch according to Lintner" persist to this day. Somewhat earlier the disappearance of the iodine-staining property of starch was being used as a criterion for studying hydrolysis. This method for the evaluation of *dextrinization* was outlined as a quantitative procedure by Roberts (23) in 1881 and popularized by Wohlgemuth (24) nearly 30 years later. The *liquefaction* of starch pastes by amylase preparations must have been one of the first actions observed. Through the years many methods for evaluating starch liquefying activity have been proposed but apparently few have the accuracy necessary for quantitative evaluation —a fact readily understood when the difficulties of preparing satisfactory substrates and devising adequate techniques are realized. Two of the more satisfactory current methods will be discussed later.

The methods developed for quantitative evaluation of amylases logically may be divided according to the three uses: liquefaction, dextrinization, and saccharification. Such methods for evaluating enzymes should not be confused with methods customarily used in the plant to test for the desired degree of starch degradation. These latter techniques have been developed primarily to measure a substrate change and not the exact activity of the enzyme present. They may take such forms as the approximate measurement of viscosity during liquefaction of the starch to a point previously found satisfactory for the purpose, or they may involve specific gravity measurements to determine whether or not saccharification has proceeded to a desirable degree. A difference between two amylase preparations may be noted but no attempt is made quantitatively to evaluate the preparations on an enzyme basis. Such methods are, however, of great value in plant practice. It is common knowledge that in certain processes the use, let us say, of twice as much enzyme does not result in twice as much conversion in the same time. It becomes necessary then to determine by practice either the optimum amount of enzyme to use under plant practice or, if a decrease in reaction time is desired, to determine the influence of enzyme concentration under actual working conditions. There are few data available which relate amylase concentration to the production of a desired commercial product, although such investigations appear to be highly desirable.

The following discussion of the specific reactions of amylases will be confined to the changes brought about by the action of these enzymes on gelatinized starch and, for simplicity, the action on starch giving a blue color with iodine. No attempt will be made to discuss the relative action on various starch fractions nor the light which enzyme studies has thrown on starch structure. The theoretical aspects of amylase action as related to starch structure have received extensive development by several workers, particularly Myrbäck, Hopkins, and K. H. Meyer, and reviewed recently in detail by Myrbäck (25) and Hopkins (26). The reader is referred to these for supplemental material on the action of amylases.

b. Specific Actions of Amylases. Liquefaction. The decrease in the viscosity of starch pastes under the action of amylases has wide application in industry. The exact nature of the changes that take place during this liquefaction, however, is not entirely clear. It is well known, for example, that a viscous paste of gelatinized starch can be "liquefied" to a considerable degree simply by vigorous beating. The only apparent change during this process is a disruption of the swollen starch granules. Under the action of a liquefying amylase the change is very similar. During the first stages of liquefaction neither dextrinization nor the production of reducing groups can be detected except with difficulty. This has led some to believe that true enzymic starch liquefaction is nothing more than a reduction of the starch aggregates to monomolecular size. Following this there would be, of course, a progressive splitting of the molecules resulting in dextrin formation and sugar production. During this secondary process a further pronounced reduction of viscosity would occur. On the other hand, the drop in viscosity could quite easily be attributed to the primary splitting of the starch molecules. This initial cleavage likewise would result in an inappreciable apparent dextrinization and in an insignificant production of reducing groups. Probably the most logical concept is that starch liquefaction consists of a reduction of starch aggregates to single molecules followed by a splitting of these molecules into dextrins of a high degree of complexity. It should be kept in mind, however, that this process is characterized by a very low production of reducing groups and an inappreciable change in the typical blue starch-iodine color of those starches originally staining blue with this reagent.

The kinetics of the liquefaction of starch by amylases apparently differ somewhat from those operative in dextrinization and saccharification. Over no range is there a linear relationship between the quantity of enzyme and the decrease in viscosity. This is not necessarily a failure of the general law relative to the early action of hydrolytic enzymes, but it is an indication that the reaction is of a different nature, and it may be that under experimental conditions the substrate-enzyme relationship necessary for this condition is not achieved. However, it does hold that with a given substrate and under limited conditions a doubling of the amylase quantity will cause a reduction to a desired viscosity in half the time.

Starch liquefaction typically is brought about by an amylase of the α type, and it may almost be taken for granted that, if an enzyme product is labeled α-amylase, it will have potent liquefying potentialities. As a matter of fact one of the most accurate methods suggested for measuring starch-liquefying ability is described as a method for the "determination of α-amylase" (27). Two methods have some current popularity in America for the quantitative measurement of starch-liquefying power. One of these was developed by Jozsa and Gore (29), refined by Jozsa and Johnston (27), and expanded recently by Landis and Redfern (28). This method depends on the preparation of a standard starch substrate from potato starch and the use of a pipette viscosimeter. By careful gelatinization and beating, the viscosity is adjusted to that stipulated. Enzyme is then added and after a definite reaction time the viscosity is again measured. The results are related to a standard curve, and enzyme activity is calculated essentially at zero time of reaction. The unit values proposed for amylase content are designated "liquefons."

Another of the methods which has proved reliable is that proposed by Blom and Bak (30). In this method it is not necessary to adjust the viscosity of the substrate within as narrow limits as those stipulated by Jozsa and Gore (29). A pipette viscosimeter is also used but it is of smaller size and with a capillary inserted. Amylase activity is calculated from the time required to achieve a drop in viscosity from a stipulated point to another half as great. Both this method and that of Jozsa and Gore (29) have proved reliable in the hands of experienced operators and appear to be satisfactory for the purpose of evaluating the potential starch-liquefying power of an amylase preparation under conditions conducive to enzyme stability.

1. *Dextrinization.* Most starches give a characteristic blue color when treated with a solution of iodine in potassium iodide. As enzymic splitting of starch occurs, carbohydrate molecules of less complexity result, and this is reflected in the color given with iodine. Dextrins of a low degree of complexity, approaching in characteristics the simple sugars, fail to give any color whatever when treated with iodine. This point has been called the achromic point. Between the two extremes of blue and colorless a range of

colors dependent on dextrin complexity and presence or absence of residual starch is found. These colors visually range through the purples, the reds, and the browns. It may be seen that dextrinization is a complex process, since the end-products are of considerable variability. For the present purpose, dextrinization will be considered as the production from starch of sugar polymers of lesser complexity than starch. These polymers characteristically have a low viscosity relative to that of starch and a low reducing power relative to that of maltose or glucose.

When an amylase of the α type is allowed to act on gelatinized starch, the characteristic drop in viscosity described above occurs. Further action results in a progressive conversion of the substrate to products which stain purple with iodine, then red, and finally give no color whatever. Characteristically, with amylases of this type a low production of reducing groups accompanies this change. Another type of dextrinization is that characteristic of the β type of amylase (*e.g.*, the amylase of ungerminated barley). In this instance the starch is rapidly converted in large degree to maltose and a "β-amylase limit dextrin." The combined end-products stain purple with iodine. If the β-amylase used is uncontaminated by α-amylase, the reaction apparently ceases at this point, and no further degradation of the dextrin occurs. (This dextrin frequently has been designated α-amylodextrin.) This particular form of starch degradation may be termed a partial dextrinization and a partial saccharification, since maltose production is rapid but not complete and the dextrin produced is complex. A third type of enzymic starch dextrinization is that resulting from the action of a combination of α- and β-amylase (*e.g.*, barley malt). In this case a rapid splitting of the starch to products which fail to give a blue color with iodine is coincident with an equally rapid production of reducing sugars.

An unusual type of enzymic dextrin formation is the production of nonreducing crystalline (Schardinger) dextrins. These dextrins characteristically result from the action of the amylase produced by cultures of *Bacillus macerans*. Recent studies (31-32) on the action of this amylase indicate that following a sharp drop in viscosity, there is a gradual production of dextrins of the Schardinger type. These dextrins characteristically have low but appreciable reducing power. Kerr (33-34) has postulated that such dextrins are not true degradation products or "limit dextrins" but the result of a synthesis from simpler configurations. The production of "Schardinger dextrins" appears to have little if any industrial significance at the present time.

It is apparent from the above that great care must be exercised in the selection of an amylase to bring about the production of a certain desired type of dextrin. However, the reduction of starch to products which are still complex but which fail to give a blue or purple color with iodine is characteristic of all amylases except pure β-amylase. Accordingly, this technique has been the basis of one type of method for evaluating amylase activity. Obviously such technique is not directly applicable to investigations of the dextrinization of starches staining red with iodine.

Some of the first methods for evaluating dextrinization were based on the hydrolysis of starch to products which fail to give any color with iodine. This achromic point is difficult to detect, and the popularity of the method of Wohlgemuth (24) may have been due in large part to his selection of a pinkish red end-point. In essentials his method consists of the addition of varied amounts of enzyme to a standard starch substrate and appraisal of the color given with iodine after a given time under standard conditions. Quantitative evaluation is based on the amount of enzyme necessary to bring about the desired change under the standard conditions. This technique has had considerable modification. A recent modification (35) which has gained some degree of popularity in malt evaluation is based on the time necessary for an enzyme to convert starch to products which give a red-brown coloration with iodine. The standard end-point is the color given by a specified combination of dextrin and iodine. The dextrinization time is inversely proportional to the concentration of amylase, and "dextrinogenic units" (42) are calculated as the number of grams of soluble starch dextrinized by 1 g. of malt in 1 hour at 30°C. A further advantage of selecting as an end-point the red or red-brown color given with iodine is that the dextrinization measured by its use is proportional to the quantity of enzyme.

For many years, such dextrinization techniques were considered to be specific measures of α-amylase and unaffected by β-amylase. Blom, Bak, and Braae (43), Hanes and Cattle (44), and Sandstedt, Kneen, and Blish (35) independently showed that by the procedure the combined action of the two amylase components of malt is measured and it is therefore partially dependent on β-amylase activity. The latter of these workers (35) proposed a dextrinization method specific for the α-amylase present in malt; *i.e.*, the measurement of the dextrinization rate in the presence of an added excess of β-amylase. This method has proved adequate for accurate evaluation of the α-amylase activity of cereals, but its use with other amylases has not been adequately investigated. In any case, the use of an excess of β-amylases in studying the dextrinizing activity of the many preparations which contain no β-amylase would seem unwarranted.

Several modifications of the procedures of Sandstedt *et al.* (35), for α-amylase have been suggested (36-39). In addition, two procedures based on the differential inactivation of β-amylase in malt and measurement of the action of the remaining α-amylase have been proposed (40, 41).

Other methods for the evaluation of the dextrinizing or "amyloclastic" activity of amylases have been suggested, such as for example, the alcohol-precipitation procedures of Caldwell and Hildebrand (45). However, evaluation by iodine-staining properties appears to be the most accurate, the most adaptable to laboratory conditions, and therefore the most popular.

2. *Saccharification.* Starch in its unaltered form has a negligible reducing action on such oxidizing agents as iodine, potassium ferricyanide, and alkaline copper sulfate solutions. The action of amylases, and particularly certain amylases, on this substrate causes a production of compounds which have marked reducing properties. When this splitting of starch results in the production of sugars, the process involved is termed saccharification. In addition to their reducing properties, these sugars are fermentable by yeast, and this in turn forms the basis for the very extensive use of amylases in the saccharification of starchy mashes for subsequent alcoholic fermentation. The importance of this type of conversion has stimulated much research on the nature of the process.

It is generally agreed that the principal sugar resulting from the enzymatic saccharification of starch by malt amylase system and at least several other systems is maltose. However, in addition to a variety of reducing dextrins, glucose and trisaccharides have been identified (15, 25, 46). Present knowledge as to the complexity of the "amylase" systems being studied makes the interpretation of previous results somewhat difficult. For example, the identification of at least three carbohydrases in malt, α-amylase, β-amylase, and limit-dextrinase (13) and at least three in fungal preparations, α-amylase, maltase, and limit-dextrinase (12, 15, 47) indicates that some of the products of "amylase" action may result from the action of carbohydrases other than amylase. At any rate, it has been, and to a considerable extent still is, the practice to calculate the sugars produced as maltose and append the phrase "calculated as maltose" to indicate uncertainty as to the exact nature of the products. This difficulty can be circumvented to some extent by evaluation of sugar produced by means of fermentation procedures (25, 47-50). Routine procedures can be applied, if desired, in such a fashion as not only to evaluate the amount of fermentable sugar produced but also to differentiate between the types of sugar present.

It is usually the case in industrial saccharification that a high degree of conversion of starch to sugar is desired. In such a conversion the kinetics of the reaction are of the two-phase nature described earlier in this chapter. In the first of these, occurring when the ratio of substrate to enzyme is high, sugar production is rapid and linearly related to enzyme concentration. The second phase is a much slower reaction and not linearly related to enzyme concentration. The foregoing holds for β-amylase action and for the action of enzyme preparations containing both α- and β-amylase. In the case of hydrolyses in which the only amylase operative is the α form, conformity to the first phase of saccharification is difficult to detect. In no range is there a linear relationship between production of reducing groups and amylase concentration. This is not surprising when it is considered that there is a complete disappearance of whole starch from the reaction medium before appreciable sugar production can be detected, the majority of saccharification being due not to a direct action on starch but to a further splitting of dextrins.

In addition to the two phases of the saccharification process, there are at least three main types of starch saccharification by amylase. One of these is that well known from observations of barley malt. A rapid dextrinization occurs accompanied and followed by a rapid production of maltose. In the later stages, the reaction slows down, but sugar production proceeds steadily until the extent of conversion approaches complete degradation of the starch to sugar. This reaction is typical of the combined actions of α- and β-amylase. A second main type of saccharification is that performed by the amylase typical of ungerminated barley, β-amylase. Saccharification is rapid and linearly related to amylase concentration in the first phase. Sugar production slows down in the later stages and terminates when some 60% of the starch has been converted to maltose (with pure β-amylase and at least several of the starches this termination coincides almost exactly with the 60% level). If the β-amylase is uncontaminated with α-amylase, this limit dextrin, at least in so far as its iodine-staining properties are concerned, remains apparently unchanged for months.

The specific action of β-amylase on starch and on starch fractions is described by Myrbäck (25) and by Hopkins (26). In addition, the report by Cleveland and Kerr (51) and by Swanson (52) that β-amylase completely degrades one molecule of amylose before attacking any other chain appears to be of prime significance from the standpoint of enzyme-substrate affinities and the mechanism of enzyme action.

A third type of saccharification is that typical of preparations designated as α-amylases. Since most amylases appear to be of this type the reaction is important. The picture has been somewhat confused due to the use of unpurified or partially purified enzyme systems. Although these systems have been of the α-amylase type and demonstrably free from β-amylase, there was no assurance of freedom from other glucosidases such as maltase and limit-dextrinase. Recent work with purified enzymes and especially with crystalline amylases is doing much to clarify the concept. For example, the data of Bernfeld and Studer-Pécha (53, 54), and of Bernfeld and Fuld (55) indicate that the crystalline α-amylases are all very similar in their qualitative action on starch but differ in quantitative action. Apparently, these differences come about by differences in the affinities of the enzyme for the substrate and particularly for the products of the primary stage of hydrolysis. Thus, a fairly clear pattern of behavior for typical α-amylase may be laid down.

The α-amylases are thought of as starch liquefying and dextrinizing enzymes and justly so, since their early action is predominantly of that nature. However, small amounts of fermentable sugar, in addition to reducing dextrins, are produced from starch during the dextrinization process, and following the attainment of the dextrin stage a steady production of sugars occurs. The extent of this post dextrin saccharification depends primarily on the affinity of the amylase for the substrate (25, 26, 53, 54, 56). The conversion to fermentable sugar may reach quite high levels providing the enzyme is not inactivated on standing and providing sufficient time is allowed for the slow reaction to progress. These variables, in addition to the tendency of the substrate to age and become difficultly hydrolyzable during the reaction (53, 54), make it difficult to establish the exact limit of conversion by α-amylase. However, for all practical purposes, the production of fermentable sugar from starch by *pure* α-amylase is not an important industrial characteristic.

Of the commercial amylase preparations only "bacterial amylases" appear even to approach the characteristics of a pure α-amylase (50). Other types of microbial, animal, or malt amylases, including those predominantly of the α-amylase type, usually have accompanying supplemental carbohydrases varying from traces to substantial quantities. As early as 1941 Kerr and coworkers (57, 58) pointed out the significance in saccharification by fungal amylases of accompanying non-amylase glucosidases. The significance of relatively trace amounts of β-amylase not measurable by customary methods on the action of sorghum malt α-amylase has been demonstrated by Kneen (10). It is readily appreciated that the presence of traces of other amylases, of limit-dextrinases and of maltase may account for many of the discordant results in the earlier studies on the action of α-amylase.

Methods for the evaluation of the saccharifying power of an amylase preparation, such as those devised to evaluate malt, have had one characteristic in common: they have been designed so that Kjeldahl's law of proportionality (21) is followed. In other words substrate concentration, time, and quantity of enzyme are adjusted so that starch conversion does not exceed 40%. They are then the same in principle. Standard conditions relative to time, temperature, pH, and substrate concentration are followed, and the sugar production is determined either by the reducing properties of the products or by other means such as optical properties or fermentability. If saccharification is calculated in terms of sugar production, the products formed usually are grouped and calculated as maltose. So long as the appropriate conversion limit is observed, the unit values used to designate enzyme activity are equally reliable. For example, when the regular Lintner method and the rapid 15-minute method of Kneen and Sandstedt (59) are used to determine the activities of the same series of malts, the relationship between "degrees Lintner" and "Kneen-Sandstedt units" is a linear function, and either "unit" may be arrived at by applying a factor to the other. It is apparent then that the method used will depend on custom and convenience. The limitations of prevalent methods for measuring saccharifying activity should be kept in mind. By those such as the Lintner or the Kneen and Sandstedt (59) technique the combined saccharifying activity of all the amylases present is measured and this activity is measured only in the early stages of starch saccharification. It is only recently that an accurate evaluation of the amount of saccharification attributable to the α and β components of malt has been possible. The development of a method (59) for the determination of β-amylase activity in the presence of α-amylase and its use with barley malts (42, 59) have shown that *on the average* 85% of the saccharifying activity of a barley malt is due to β-amylase and that the other 15% is due to the α component. Further, in the procedure of a manufacturing plant a type of saccharification extending over several days may be practiced. In this instance, *i.e.*, the production of alcohol from starchy mashes, the saccharification need not be the very rapid conversion characteristic of the action of barley malt on gelatinized starch but only one supplying fermentable sugar in amounts adequate for yeast capacity. For such processes the "Lintner value" of an enzyme product may be of little value; the utility of the product may depend on the content of α-amylase and other enzymes not adequately measured by a method largely dependent on β-amylase activity.

The evaluation of amylolytic activity whether it be based on saccharification, dextrinization, or liquefaction is a necessary adjunct to standardization of preparations and to the division of such preparations into the various utility groups. Such evaluations serve, of course, only as guides in the selection of an enzyme for industrial use and the prediction of its value therein. It should be emphasized again that until adequate correlations are worked out between known amylase concentrations and properties and the manner in which these are operative under plant conditions, final evaluation can be made only by trial.

4. Properties of Amylases.

a. General Properties. It is not within the scope of the present treatment to deal extensively with the development of the present views regarding the nature and the kinetics of action of amylases. Present evidence indicates strongly that amylases either are protein entities or are specific groupings intimately associated with specific proteins. From Kirchhoff's identification of the characteristic action as associated with the "gluten" of cereal grains, through Osborne's researches (60-61) and the multitude of papers following, the properties of this enzyme have appeared as those typical of proteins. All amylases are irreversibly inactivated by high concentrations of acid or alkali, and by boiling temperatures. They do not pass through cellophane dialysis membranes. They are soluble in water or very dilute salt solutions and in dilute ethanol solutions. At higher levels of salt (ammonium sulfate) and higher concentrations of ethanol they are precipitated in forms that are active when redissolved in water.

The crystallization of a number of amylases in sufficient quantity for characterization confirms the previous findings with partially purified materials that the amylases are proteins. The first amylase crystallization apparently was by Caldwell *et al.* (62) who reported isolation of pancreatic amylase crystals in small yield. Recently this crystallization has been repeated for hog pancreatic amylase by Meyer and collaborators (63, 64) and extended to human salivary amylase (65), to *Bacillus subtilis* amylase (66), and to human pancreatic amylase (67) by the same group of workers. These crystalline proteins while differing, for example, in the manner of crystallization, in solubilities, and in optimum pH for activity are remarkably similar in their actions on starch and also closely comparable in action to purified malt α-amylase (55). They do differ in their affinities for the substrate (54) which differences lead to quantitative variations in the manner of hydrolysis particularly in the post dextrinization stage.

Crystalline β-amylase from sweet potatoes has been isolated and described by Balls and collaborators (68, 69) and crystalline malt α-amylase has been isolated by Schwimmer and Balls (70). Here also additional evidence is supplied that the amylases are protein entities and are not dependent on prosthetic groups for activity.

The molecular weight of hog pancreas amylase is thought by Meyer and collaborators (64) to be somewhat less than 20,000. On the other hand, based on ultracentrifugal studies, Danielsson (71, 72) reports molecular weights of 45,000 to 54,000 respectively for pancreatic amylase and malt amylase.

The activity of any amylase is dependent on a number of factors. The rate of reaction may or may not be influenced by various salts, protein cleavage products, lipoids, etc., and by other substances which have been classified as specific activators and inhibitors. It is becoming increasingly apparent that many of the so-called "activators" function either to increase the solubility of the amylase or to increase its stability. The stabilizing action of calcium ion is a good example.* The principal effect of this ion on α-amylase is to increase the stability of the enzyme (73, 75). This stabilizing action is so pronounced that purified malt α-amylase is stable only when calcium ion is present (70), and this must be taken into account in the crystallization of the enzyme in an active form. Di Carlo and Redfern (74) found that the calcium ion is a necessary stabilizing agent also in the purification of *Bacillus subtilis* α-amylase. As a further instance of the complexity of these stability factors it has been shown (75) that the depressing effect noted for calcium ions on β-amylase, the other amylase component of malt, is to decrease its stability.

It is commonly accepted that the end products of amylase action have an inhibitory effect on the further action of the enzyme. Other inhibitory substances of a more specific nature have been described. Examples are the insoluble substances present in germinated buckwheat (76, 77), the water soluble, protein-like substance (78, 79) present in the endosperm of wheat and rye, and the water soluble high molecular organic acid present in certain sorghum grains (80). These two latter substances give a reversible inactivation of certain amylases (79, 80). The one in wheat appears definitely to be a protein (81) and its mode of action is non-competitive (82). Interestingly enough, the wheat inhibitor is effective only on animal amylases and on one of the bacterial amylases (79), whereas the one in certain of the sorghums inhibits all amylases with the exception of those derived from the growth of fungi (80). Both the inhibitors appear to contain the indole nucleus as an essential constituent. This would appear to be significant in view of the report by Eyster (83) that certain plant "auxims", *e.g.*, indole propionic acid, inhibit the action of amylases. The specificity of these inhibitors has suggested their use in differentiation and characterization of the amylases (50, 84).

From an industrial viewpoint, perhaps the most important characteristics of amylases, aside from the type of conversion they catalyze; are those relating to the influences of pH and temperature. All amylases have pH optima for their actions. The pH optimum for any specific amylase will depend on the duration and temperature of the reaction. This apparent anomaly results from the difference between the pH optima for activity and for stability. For example, malt α-amlyase is most stable at a pH close to 7.0, but the optimum for activity at 30° C. is not above pH 5.0. If the reaction temperature is raised sufficiently to cause a progressive inactivation of the enzyme, the apparent optimum pH will be raised accordingly. In other words, the apparent optimum activity is a compromise between the actual optimum and the pH value at which the amylase has the greatest stability. Similarly, under conditions contributing to instability, the longer the reaction time, the closer to neutrality the pH optimum for activity becomes. In industrial utilization therefore it is important that an amylolytic conversion be carried out at a pH which is optimum under the particular conditions operative and not necessarily at a pH which may be optimum under any other set of conditions.

---

*For the effect of calcium salts on the activities of α- and β-amylases see, for example, the specification of Kerr and Trubell, French Patent 863,043 (1940) to Corn Products Refining Co. (Ed.)

The relationship between amylase activity and reaction temperature likewise is of utmost significance in industrial application. In low temperature ranges, for example between 20 and 30° C., the rate of conversion is approximately doubled by a rise in temperature of 10° C. As the temperature is raised to higher levels, the increase in reaction rate with increase in temperature diminishes until a point is reached at which further increase in temperature has no apparent influence. In this region the rate of irreversible loss of amylase exactly balances the increase in activity of the remaining amylase. Further increase in temperature causes a more pronounced loss of enzyme and, eventually, complete destruction of the amylolytic power of the preparation. Since loss of enzyme activity is a function of time and pH as well as of temperature, it is obvious that these factors likewise must be taken into account. An optimum temperature for amylase activity does not exist, merely a temperature which, under the particular working conditions, will cause the most rapid completion of the change desired.

Amylases from different sources have different degrees of thermostability. The rise in popularity of bacterial preparations may be attributed almost entirely to the thermostable nature of some of these amylases in the combined gelatinization and degradation of certain starches with high temperatures of gelatinization. Under other conditions when resistance to thermal inactivation is not as critical a factor, an amylase deficient in this respect can be utilized. For any amylase of the α type, *i.e.*, primarily a starch-liquefying and dextrinizing enzyme, the resistance to thermal inactivation can be increased by certain adjustments in working conditions. The more important of these are the adjustment of the pH to one more nearly approaching the optimum for stability, and the addition of calcium ions. It thus becomes apparent that an amylase preparation of low thermostability in the natural state may be made to approach in this regard one of higher stability. Two types of stability are apparent: the inherent stability of an amylase, and the stability conferred upon the enzyme by substances which either occur naturally in conjunction with the amylase or are supplied as adjuncts.

b. Specific Properties. As discussed above, it becomes somewhat difficult to state the properties of a specific amylase without elucidating exactly the conditions under which such properties are measured. Accordingly, the properties of the various amylases will be described in rather general terms but with the intent of differentiating clearly between those from different sources. Very few adequate comparative studies have been reported, and some of the specific data available in the literature are contradictory. The conclusions expressed are those arrived at by examination of much of the literature and from data, some of which are unpublished, obtained by the writer and collaborators. The discussion will be divided into considerations of the three principal groups of amylases, those from higher plants, those of microbial origin, and those from animals. No attempt will be made to deal with all the amylases; attention will be directed to those which, at the present time, have potential industrial significance.

1. Amylases of Higher Plants. Amylases of this group were perhaps the first used industrially. Their properties will receive more attention in the discussion than may seem warranted but there are several reasons for this. Extensive research has been carried out on this group, particularly on the amylases of barley malt; hence there is accurate and abundant information. Barley malt, among others, contains the two amylase components, α- and β-amylase, and therefore lends itself particularly well to a discussion of these components. Since all amylase properties are of relative degree, moreover, it is convenient to use the barley amylases as standards for comparison.

For the present purpose, the higher plants may be divided into two groups, one dealing with the cereals and the other embracing plants outside this group which may have industrial significance. The cereals may be divided into two subgroups, the malts and the ungerminated grains.

Cereal Malts. The best known member of this group is barley malt. Since its amylase system is composed of two components, α-amylase and β-amylase, the starch-degrading properties of barley malt are those of the combination of these components found in the particular sample. One malt may be high in β-amylase and low in α-amylase, another just the reverse. Of the three main functions of amylases, only one (42), starch liquefaction, appears to be influenced by a single component, α-amylase. Dextrinization is greatly influenced by both components, and, while saccharification as customarily measured is largely dependent on β-amylase activity, a minor rôle is played by the α component. The properties of these two amylase components vary widely and will be discussed separately. Varying nomenclature has been applied. Wijsman (85) in 1890 made a clear separation of the two and called them "dextrinase" and "maltase" to describe their dextrin-producing and maltose-producing properties. (The "maltase" referred to in the older literature as a diastatic agent should not be confused with the maltose-splitting enzyme.) Kuhn (86) in 1925 proposed the nomenclature of "α-amylase" and "β-amylase" and indicated that they produced respectively α- and β-maltose. At about the same time Ohlsson (87-88) proposed that they be called "dextrinogenic amylase" and "saccharogenic amylase." At present the terms α- and β-amylase are the most popular, though customarily they are not used in the sense implied by Kuhn. Rather, α-amylase is thought of as the starch-liquefying and starch-dextrinizing enzyme, β-amylase as the typical starch-saccharifying enzyme. Characteristically α-amylase is the amylase of "activity," β-amylase the amylase of "dormancy." In general it appears that if amylase is synthesized as a result of active growth, the α form predominates; if laid down in conjunction with starch in reserve or storage organs the β form predominates.

Barley Malt α-Amylase. This amylase acts on "raw," native starch (42), and readily liquefies and dextrinizes gelatinized starch with a minimal early production of sugar. Essentially the action is a breaking of the molecule into large fragments (89). Following the initial rapid splitting of the molecules in which some 15 to 20% of the starch linkages are broken (25), a slow production of smaller dextrins and fermentable sugars results with the eventual degree of conversion depending on time, stability of the enzyme, affinity of the enzyme for the substrate, and physical changes in the substrate due to aging. The pH optimum for activity at near room temperatures is in the neighborhood of pH 5.0 and usually is somewhat below 5.0. The amylase is relatively unstable, especially in dilute solutions, and its stability is remarkably enhanced by the *presence* of calcium ions. Inactivation is more rapid at a low than at a high pH and the pH optimum for stability is approximately 7.0. It is resistant to surface denaturation and has a fair degree of thermostability. At pH 6 to 7 and in the presence of calcium, loss in activity at 70° C. is slow. α-Amylase is soluble in water or the very dilute salt solutions resulting from extraction and is rendered more soluble by the peptizing action of either dilute salt solutions or the proteolytic enzyme papain. More concentrated salt solutions (*e.g.*, 25 to 35% ammonium sulfate) cause precipitation. α-Amylase is readily soluble in dilute ethanol solutions but precipitates at levels in the neighborhood of 60% ethanol.

Barley Malt β-Amylase. This amylase does not act on most native starch granules and liquefies and dextrinizes gelatinized starch inefficiently. Saccharification of gelatinized starch is rapid but incomplete, ceasing with many starches when conversion to about 60% maltose and 40% "limit dextrin" has occurred. Saccharification is achieved by the breaking away of single maltose molecules from the ends of chains or of chain branches (89). The pH optimum for activity, near room temperatures, is about 4.5. This component is relatively stable at room temperature, especially in the *absence* of calcium ions. Inactivation is much more rapid at high than at low pH values and the pH optimum for stability appears to be in the neighborhood of 5.0. It is sensitive to surface denaturation and has a low degree of thermostability. Thermal inactivation is largely completed after a few minutes at 70°C. Solubility characteristics are similar to those given above for α-amylase except that higher concentrations of ammonium sulfate or of ethanol are required for complete precipitation and dilute salt solutions or papain-like enzymes effect a greater release into solution. A certain degree of characterization of the enzyme has been achieved (90, 91) and it appears that free sulfhydryl and free tyrosine groups are essential to its activity while free amino groups probably are not essential.

The above description of the components of barley malt amylase indicates the reasons for the versatility of this product. Malts of the other cereals may be divided into two groups. The first group is similar in amylase composition to barley malt.

Malts of Wheat and Rye. These malts have both α- and β-amylase as components of their starch-degrading systems and in somewhat similar amounts and relative proportions. The respective components of these malts are similar in their essential properties to the α- and β-amylases of barley malt. Minor differences do occur but may be due to a variation in substances accompanying the amylase in extracts rather than to a fundamental difference in the enzyme. For example, barley α-amylase appears to have greater thermostability than wheat α-amylase, whereas the relative thermostabilities of the β components are just reversed (75). As a consequence of the similarity in amylase composition, the malts of barley, wheat, and rye, exhibit similar actions in respect to starch liquefaction, dextrinization, and saccharification.

Malts of Oats, Sorghum, Maize, and Rice. The three cereal malts discussed above have in common the presence, in addition to α-amylase, of considerable quantities of β-amylase. Those now treated may develop similar levels of α-amylase on germination, but all are characterized by a deficiency of β-amylase (92). In fact, this component is present in such small amounts that its activity usually does not become apparent when macro-methods of evaluation (22, 59) are employed; however, the use of micro-techniques permits an estimation of the levels present (92, 93).

The α- and β-amylases of oats, sorghum, maize, and rice appear to be quite similar in properties to the corresponding amylases of barley malt with, of course, minor variations. The pH optima for activity, thermal reactions, and solubilities are comparable. The mode of starch degradation of the whole malts is similar to barley malt α-amylase in the early stages, up to the point of complete conversion of the starch to low molecular dextrins. Following dextrinization, sugar production proceeds at a rate faster than that characteristic of α-amylase to a degree governed by the level of β-amylase present. Since wide varietal differences may occur, both in α-amylase activity and in the micro-quantities of β-amylase present (unpublished data), the rate of this postdextrinization saccharification is a characteristic of the particular malt used.

It has been well known that the malts of such cereals as sorghum and maize are low in the type of saccharifying action typical of β-amylase activity. Nevertheless, sorghum malt has been used with success in India (94) and in South Africa (5) for the conversion of starch to fermentable sugar. It appears that the presence of a large proportion of β-amylase in a malt (high Lintner value), while frequently desirable, is not an essential feature. Further, the wide variations in the amylolytic properties of malts emphasizes that the common term "malt," as used to characterize a certain type of amylase, is a misnomer.

It is appreciated that the starch-degrading properties of malts are not the only characteristics of significance in industry. By far the greatest percentage of malt is employed in the production of alcoholic beverages. In these instances, such qualities as flavor receive necessary consideration. However, if the cereals are evaluated solely on the basis of amylase development during malting, certain ones have more value than others. Among the "cool climate" cereals, barley malts appear to average somewhat higher in β-amylase activity than malts of the others. Oat malt is very low in this respect, and the malts of wheat and rye lie close to that of barley. Wheat appears to have superior ability for the synthesis of α-amylase during germination and again oats are low. Of the "warm climate" cereals, certain varieties of sorghum, particularly "kafir," appear to be superior in ability to synthesize α-amylase, and the malts may achieve high levels of this component. Selection of a malt to perform a definite type of starch degradation should be based on the content of the desired amylase unless other properties preclude its use.

Ungerminated Cereals. Ungerminated cereals invariably have lower amy lolytic powers than the malts produced by germination. As with the malts, they may be divided into two groups, based on the content of β-amylase. Most appear to have microquantities of α-amylase present even in the well ripened grain (92). The cereals that produce malts having high levels of β-amylase activity are relatively well supplied with β-amylase in the ungerminated state; those having a deficiency of β-amylase activity when malted show very low activities before germination. Accordingly, barley, wheat, and rye contain an active β-amylase in considerable quantities, and the starch-degrading properties of their extracts are those characteristic of β-amylase. In addition, traces of α-amylase are present. A large proportion of the amylase contained in the grains is difficultly soluble, and extraction is markedly increased by the use of dilute salt solutions or papain-like proteolytic enzymes. However, even if the amount of α-amylase extracted is doubled or tripled by these procedures, it still is relatively insignificant and contributes little to the typical β-amylase degradation of starch performed by such extracts.

Extracts of the ungerminated grains of sorghum, maize, and rice are practically inactive from an amylase standpoint. Though ungerminated oats have appreciable β-amylase activity, it is limited to about one-tenth of that found in barley or wheat (92). All show the very low, but measurable, liquefying and dextrinizing ability characteristic of the traces of α-amylase usually present; when compared to the α-amylase activities of the malts this is of negligible degree.

Higher Plants Other Than Cereals. It is evident that practically all plants, as well as animals, utilize amylase activity in their physiological processes. However, in only certain instances is the concentration of these enzymes sufficient to offer industrial possibilities. With the higher plants such concentration, if present, occurs in the storage organs. Two sources of amylase have been suggested: the seed of soybeans and the underground storage organs of the sweet potato plant.

Newton and coworkers (95, 96) have conducted investigations of the properties of soybean amylase. They characterize the enzyme as β-amylase inasmuch as it has starch-saccharifying powers similar to those exhibited by β-amylase from cereals. Their data indicate that the amylase is present in considerable quantities in the ungerminated bean and can be extracted with water and concentrated by precipitation with 65% ethanol. The suggestion is made that soybean β-amylase has a higher degree of thermostability than cereal β-amylase. In unpublished investigations the writer has found that soybean extracts contain small amounts of an α type of amylase in addition to the typically saccharogenic amylase. This indicates that, as with the cereals, a procedure for differentially inactivating the traces of α-amylase must be employed before the amylase may be used in studies necessitating "pure" β-amylase.

The fact that the sweet potato is rich in diastase was noted by Gore (97) over 20 years ago. Giri (98, 99) made extensive studies of this amylase and found it to be very similar to barley malt β-amylase in its action on starch and in its properties. The action of this amylase has some value in the preparation of sweet potato sirups.

2. *Microbial Amylases.* The discussion of these will be divided into considerations of the properties of amylases produced by the growth of the fungi and of the bacteria.

Fungal Amylase. The fungal amylase most widely produced and therefore best known is that resulting from the growth of appropriate strains of *Aspergillus oryzae*. This amylase is very similar in starch-degrading properties to malt α-amylase with the exception that postdextrinization saccharification is more rapid. The characteristic high liquefying power is present, and dextrinization is rapid and coincident with a low production of sugars. The later stages of the action on gelatinized starch are marked by a steady production of fermentable sugars. The fact that this saccharification proceeds to high levels of starch conversion and is sufficiently rapid to meet yeast requirements is evidenced by the efficiency of some fungal amylase preparations in alcohol production. The pH optimum for activity is about the same as that of malt α-amylase; namely, 5.0. Calcium acts as a stabilizing factor. Solubilities in salt solutions and ethanol are similar to those of the other amylases. Fungal amylases characteristically are less resistant to thermal inactivation than malt α-amylase, and therefore their apparent temperature optima for activity are lower. At 70°C. the loss in activity is very rapid.

The fact that fungal enzyme systems are efficient in the saccharification of starch to fermentable sugars, notwithstanding their apparent deficiency in an amylase of the beta type, indicates either that the α-amylase of these systems is unique or that accompanying non-amylase carbohydrases markedly modify the course of the reaction. This was observed by Kerr and coworkers (57, 58) but it is only recently that extensive breakdown of the carbohydrase systems of a number of molds has been achieved. Le Mense *et al.* (100), Corman and Langlykke (15), and Gates and Kneen (47) have presented data for both the α-amylase and maltase activities of enzyme systems produced by culturing a wide variety of fungal types ranging through *Aspergillus, Rhizopus, Penicillium*, etc., with particular attention to the *Aspergilli* It has been shown that the activities of the two enzymes, α-amylase and maltase, vary quite independently of each other. Most important is the observation (15, 100) that, providing minimal concentration of α-amylase be present, the alcohol producing capacity of the systems was dependent on the maltase activity. This result, combined with the finding that mold enzyme sytems contain a third enzyme, "limit-dextrinase" (12) readily explains why an enzyme system which is typically α-amylase in some application functions adequately as a saccharifying agent over longer periods of time. The characterization of fungal α-amylases must await separation of these as entities free from accompanying non-amylase enzymes.

Bacterial Amylases. The commercially available amylase preparations resulting from the culture of bacteria appear to be of the alpha type, with starch degrading properties similar to those of malt α-amylase (43, 50, 101). In other words they are typically starch liquefying and dextrinizing enzymes with low saccharifying power. Like other α-amylases, the activity is stabilized by calcium ions. Solubility characteristics are similar to those of other amylases. The pH-optimum for activity is somewhat higher than that for malt α-amylase, being in the region pH 6.0 to 7.0 (102). The industrially available bacterial amylases, those produced from selected strains of *Bacillus subtilis,* have a greater degree of thermostability than any other of the commonly used amylases and may be employed with little loss of activity at temperatures above 70°C. This thermostability, resulting in a high apparent temperature optimum for activity, is the most important attribute of the amylases and is the chief factor contributing to their expanding use.

The amylase from certain commercial strains of *B. subtilis* has been characterized as a protein (66). Di Carlo and Redfern (102) found that there is no evidence for the existence of a bacterial amylase coenzyme. In addition, these authors demonstrated a remarkably high degree of thermostability for the enzyme and a stability to pH values as high as pH 10.8. In a further study (74) they showed that the presence of sulfhydryl groups is necessary for activity, that amino groups appear to be essential, and that the presence of calcium ions is necessary for stability.

Although major attention has been directed to this particular type of bacterial amylase, presumably because the strains of *B. subtilis* producing it are very efficient in production (50), other types have been investigated. Effront (103) found that it was possible to produce bacterial enzyme systems of much greater saccharifying potential than that characteristic of purified α-amylase. Kneen and Beckord (50) studied the amylase systems produced by *B. macerans, B. polymyxa,* the "saccharifying" *B. subtilis* and the "non-saccharifying," commercial type of the latter. They found that the commercial type of *B. subtilis* was the only one that approached a pure α-amylase.

The "saccharifying" *B. subtilis* produced an enzyme system strong in post-dextrinization saccharification and the *B. polymyxa* system was similar to the malt system in giving saccharification both during and after dextrinization. An unexpected finding was that the highly active preparations from *B. macerans* cultures not only gave starch hydrolysis in the customary fashion for this system to Schardinger dextrins, but also carried on a slow and steady post dextrinization saccharification to give substantial quantities of fermentable sugar.

Additional studies on bacterial amylases include that of Hockenhull and Herbert (104) showing that *Clostridium acetobutylicum* produces an enzyme system composed of amylase and maltase, that of Rose (105) describing the *B. polymyxa* system, and that of Bois and Savary (106) with *B. phytomonas* and *actinomyces.* In addition to their studies with *B. macerans*, Tilden and Hudson (31) also investigated the enzyme system produced by *B. polymyxa.* Myrbäck and Willstaedt (107) provide further data on *B. macerans.*

The above discussion emphasizes the variety of enzyme systems that may be expected from the culture of various bacteria. The terms "bacterial amylase" and "fungal amylase" certainly must be considered as very inadequate designations since not only may these enzyme systems vary widely in starch hydrolyzing properties but also in pH optima, thermostability, and in utility, depending on the organism cultured.

3. Animal Amylases. These embrace a multiplicity of amylases: pancreatic, salivary, and the amylases of urine, blood, etc. The amylase secreted in the pancreas appears to be the one of major industrial importance and the discussion will be concerned primarily with it. The other animal amylases are similar in starch-degrading action and in properties to that derived from the pancreas.

Pancreatic amylase preparations are typical α-amylases with the usual starch-degrading properties characteristic of this enzyme. There is no evidence that β-amylase or an amylase with β characteristics is present. Accordingly, the preparations essentially are starch-liquefying and starch-dextrinizing in nature. Relative to these actions the saccharifying potential is low, with most of the sugar production occurring after dextrinization has been completed.

In common with all animal amylases, pancreatic amylase is very sensitive to neutral salts and requires their presence in solution for maximum expression of amylase activity. Dilute solutions (0.1 to 0.5%) of sodium or calcium chloride are commonly used, and it appears that such salts can truly be called "accelerators" and not just "stability factors." The nature of this acceleration is obscure. It seems probable that the colloidal suspensions of these animal amylases differ from those of other amylases perhaps in degree of protein dispersion. It may be postulated that the neutral salts act as peptizing agents in a manner to increase the solubility and activity of the amylase protein. In addition to their sensitivity to salt, animal amylases are distinguished by having pH optima for activity at somewhat higher ranges than those for the majority of the plant amylases. Under ordinary conditions pancreatic amylase shows its maximum activity at about pH 7.0.

Pancreatic amylase is of low thermostability, in this respect being at a level below malt α-amylase and at one similar to fungal amylase. As in the case of other α-amylases, calcium acts as a stabilizing factor. Other properties such as solubility in dilute salt and ethanol solutions and precipitation at higher levels of such reagents appear to be similar to those of the amylases in general.

A certain degree of chemical characterization of pancreatic amylase has been posible with somewhat purified preparations (2, 108, 109). However, recent investigations by Meyer and coworkers (63, 64, 110, 111) give the detailed characterization of both crystalline hog pancreatic and crystalline human salivary amylases. These two amylases, although differing markedly in chemical constitution, in stability, in pH optimum for activity, and so forth, appear to have identical enzymatic actions on starch. This lends further emphasis to the concept that all the pure α-amylases, when freed from contaminating substances and from supplementary carbohydrases, are remarkably similar in their starch degradation characteristics. To be sure, quantitative differences in activity persist but qualitatively they are alike. It appears that, at least from an activity standpoint, a reasonable definition of α-amylase is becoming possible, no matter what the source.

5. Production of Amylases. The chapter would not be complete without a brief discussion of the manner in which preparations of desirable amylolytic properties are obtained. In some instances, as with the storage organs of plants and certain glands of animals, the amylase need only be extracted. In other cases a synthesis of amylase is brought about; *e.g.*, in the germination of cereals and in the growth of fungi and bacteria.

a. Production by Higher Plants. As a plant approaches maturity, considerable concentrations of complex carbohydrate and of protein may be deposited in storage organs. During the progress of the deposition, an amylase may be laid down in close association with the starch and other proteins. This "amylase of storage" typically is a β-amylase. As pointed out in the preceding section, this deposition of β-amylase is not universal and may be the exception rather than the rule. The seeds of certain cereals have it in large quantities, others in traces or not at all; most legume seeds are deficient in amylase but soybeans have liberal supplies. Its presence in abundance is not evident in all underground storage organs but certain ones such as those of the sweet potato are excellent sources.

β-Amylase *relatively* free from the α form does not have a great deal of industrial importance. If such an amylase is desired, it may be extracted from appropriate sources such as barley, wheat, rye, soybeans, and sweet potatoes. Extraction is readily accomplished by the use of dilute neutral salt solution such as 1% sodium chloride. Papain may be a valuable adjunct to extraction, especially in the case of the cereals. Once obtained in solution the β-amylase may be used "as is" or concentrated and partially purified by precipitation by neutral salts in high concentration or by 80% ethanol. The precipitate is separated, dried, and handled as a stable powder. Details of a method for preparing β-amylase free from the α form are given by Kneen *et al.* (75).

When the seeds of the cereals are placed under favorable growing conditions and allowed to germinate, a rapid production of α-amylase almost invariably takes place. For those grains originally containing β-amylase there is a concurrent increase in solubility of this stored amylase caused, it is believed, by the action of proteolytic enzymes simultaneously developing. Conditions adopted for germination or "malting" of the grains are selected to suit the particular cereal. For example, the low temperatures customarily used in the malting of barley are entirely unsatisfactory for the germination of sorghum. This cereal and others of like nature require considerably higher temperatures to promote active growth.

The malting process from the grain to the finished malt includes in its essentials the selection of a desirable grain which is cleaned, steeped, or soaked to an appropriate moisture content, and then allowed to germinate either on a "floor" in compartments or in "drums" with appropriate moisture and temperature conditions and with adequate aeration, and finally dried. The process will vary, depending on whether a "brewers' malt" or a "distillers' malt" is the desired product. For example, in the production of a distillers' malt conditions favorable to the development of high amylase activity should be observed, and drying may be carried out at the relatively low temperatures necessary for the preservation of this activity. On the other hand the yield and nature of the extract are of greatest significance in the production of brewers' malt, and no attempt is made to develop and maintain an amylase activity higher than "adequate." For a complete treatment of the biochemistry of malting a standard reference such as Hopkins and Krause (112), Leberle (113), or DeClerk (114) should be consulted.

In the preceding section of this chapter it was indicated that malts vary widely in their amylolytic activities. Detailed information relative to the nfluence of the kind of cereal, the variety, and the growth environment on he malting quality of cereals cannot be included in this discussion. It must suffice to say that a malt of almost any desired amylase content and ratio of amylase components can be prepared by proper selection of the cereal and of the production conditions.

Depending on the purpose for which it is to be employed, the dry malt may be either ground and used as such, milled to produce a malt flour, or extracted. Water extracts may be used or concentrated at low temperatures to give diastatic sirups. In such extracts or sirups the α-amylase is relatively unstable, particularly when a deficiency of calcium exists. Accordingly, storage and handling of concentrated malt amylase preparations are best accomplished in the form of the precipitates obtained by neutral salt or alcohol precipitation.

b. Production of Microbial Amylases. Individual fungi and bacteria vary greatly in their potentialities for producing amylases and excreting them into the media on which they are grown. Not only do certain groups appear to have greater potentialities than others, but strains within a group vary markedly in this property. The only distinguishable difference between two such strains may be in this property. In the selection of an organism to be used in production two possibilities are apparent: A strain previously shown to be valuable may be employed, or a new strain may be isolated. If the latter method is chosen, it becomes a matter of collecting all available strains of a group known to have promise, of comparing their amylase production, and of selecting the most promising individual strains. The dependency of amylase production by any one strain on the medium employed and on the growth conditions further adds to the complexity of the process.

Fungal Amylases. These amylases customarily have been produced by the growth of selected fungi on solid or semisolid media. Only the general features of production will be given here. More detailed information may be obtained by consulting the papers of Takamine (115-6), Oshima and Church (117), Harada (118), and Underkofler and coworkers (119, 120).

Various types of media have been used for growing the fungi. The most popular material at present appears to be wheat bran. Various fungi have been used, but strains of *Aspergillus oryzae* have proved most popular. Hao and coworkers (121) prepared "mold brans" by the growth of members of the genera *Aspergillus, Mucor, Penicillium,* and *Rhizopus* on wheat bran and concluded that the better strains of *Aspergillus oryzae* were most satisfactory.

In general terms, the preparation of "mold bran" may consist of cooking a mixture of equal parts of wheat bran and water, cooling, inoculating with the fungus, maintaining at 30-35° C. with adequate aeration for several days, and finally drying at low temperatures. The whole process, including culture of the mold, preparation of the inoculum, and subsequent growth must be performed in such a manner as to minimize the possibility of contamination. Dilute acid may be added to the medium before growth; adjustment to about pH 4.0 tends to prevent the growth of bacterial contaminants. Various salts known to be necessary for satisfactory mold growth may be added to supplement those present in the bran. The time necessary for the mold to develop sufficiently for maximum amylase production need be no more than 2 or 3 days. It is generally supposed that this point is reached just before prolific sporulation becomes evident. Conditions relative to agitation and aeration during growth are critical. The process may involve a period at rest, with no aeration, to permit rapid growth, followed by active movement of air through the mass until maximum growth is attained (121).

The mold bran or "Koji" resulting from the above process may be used "as is" for various purposes. Extracts may be made and the amylase precipitated with alcohol, separated from the liquid, and dried (122). The water-soluble concentrates of amylase prepared by this means have wide applicability in industry. One of the better known of these concentrates is Takadiastase. In fact this name frequently has been used in a general sense to refer to purified concentrates of fungal amylase.

During recent years there has been an emphasis on the production of fungal amylases by culturing the organism submerged in aerated nutrient liquids. This received its impetus from the work of Le Mense *et al.* (100) who surveyed many fungi and concluded that certain strains of *Aspergillus niger* were well adapted to submerged culture. These strains not only produced high levels of α-amylase but also of maltase and gave liquids of utility in the saccharification of corn mashes for alcohol production. The necessary details for large scale production of the enzyme system by this procedure have been given by the above mentioned authors and by others (123-125). In essentials it consists of inoculating deep culture liquids with the desired organism and culturing with adequate aeration and control of pH and temperature until the desired activity is attained. Since the organisms are highly aerobic, very efficient aeration must be practiced. A satisfactory nutrient has been thin stillage supplemented with corn meal and adjusted in pH with calcium carbonate. The crude liquid may be used as produced or the enzymes removed and concentrated by the usual precipitation techniques.

Bacterial Amylases. In many respects the techniques used for the production of bacterial amylases are simliar to those prevalent in the production of fungal amylases. There is the same necessity for selecting a satisfactory strain of organism, for using a favorable medium and optimum growth conditions, and for minimizing contamination. Production of bacterial amylase customarily has been on liquid media and fungal amylase on solid media. That these conditions may be interchanged has been demonstrated by Le Mense and coworkers (100) for fungi and by Beckord and coworkers (49) for bacteria. Adequate aeration is necessary for both bacteria and fungi. A definite difference in culture conditions has been the use of somewhat acid media for fungi and media close to neutral for bacteria. Details for bacterial amylase production may be obtained by consulting Effront (126), Boidin and Effront (127, 128), Schultz *et al.* (129) Wallerstein (130), Beckord *et al.* (49) and Beckord *et al.* (131)

The media used for the growth of bacteria for amylase production customarily are high in nitrogen and low in carbohydrates. Soybean and peanut meals, wheat bran, and thin stillage have been used successfully to prepare such media. They may be subjected to previous hydrolysis in order partially to break down the proteins and carbohydrates. The addition of various salts may or may not be found necessary for optimum growth. The liquid is adjusted approximately to the pH of neutrality, sterilized under pressure, then inoculated with a pure culture of the bacterial strain. This liquid customarily is held in shallow trays, the depth of the layer being only a few millimeters. Aeration is carefully regulated, and the temperature is maintained at about 30°C. When growth of the organism has resulted in the maximum production of amylase, the liquid is ready for use in such form, or the amylase may be precipitated from it by the use of neutral salts or alcohol. It is customary to remove the majority of the bacteria before precipitation of the amylase. This may be accomplished by centrifuging and to some extent by techniques of differential precipitation.

Many bacteria have been investigated with regard to their amylase production capabilities. Note, for example, the work of Peltier and Beckord (132) and Kneen and Beckord (50). The organisms of greatest value have been found in the *Bacillus subtilis* group. Many, perhaps most, strains of *B. subtilis* appear to be deficient in this property but occasionally a strain of great potency is isolated and used for commercial production. Isolates made from "ropy" bread have proved to be very efficient in amylase production (50, 132) and in addition produce the type of amylase with low saccharifying potential and high thermostability characteristics of the commercially available materials. All other strains investigated, from sources other than "ropy" bread, produced a carbohydrase system of several enzymes rather than just α-amylase and, in addition, were less efficient in the quantity produced.

Present information (49, 50, 131, 132) indicates that from bacteria a wide variety of amylase systems, or carbohydrase systems can be produced by cultures grown on a variety of media. The organism selected for use will be determined by the type of enzyme system desired, and the prevailing economics in regard to cultural conditions.

c. *Production of Animal Amylases.* This is a phase of amylase production with which the writer has not had personal experience. The process appears to be relatively simple and only slightly different in essentials from the extraction of amylase from the higher plants. Apparently the pancreatic glands of slaughtered cattle, hogs, or horses are most commonly used. Crude preparations may be made by drying the mass of glandular material in a vacuum and removing the fat. For products of greater purity and water solubility the fluids may be expressed by appropriate means and the amylase precipitated by neutral salts. The resulting precipitate is centrifuged, and then washed and dried to give a light colored powder of high starch-liquefying and dextrinizing powers. Activating substances such as salts or protein degradation products may be added.

Sometimes in the marketing of amylase concentrates a filler is used. Frequently this filler is a neutral salt such as sodium sulfate, but with products designed for special purposes it may be sugar, starch, or even wheat flour. Accordingly, an investigator should exercise caution when attributing properties to a certain enzyme product. For example, a product prepared by diluting a fungal amylase with wheat flour would exhibit β-amylase properties in addition to the expected α-amylase activity.

6. Other Enzymes with Amylase-like Properties. It is necessary to consider very briefly a few enzymes which have or are claimed to have functions similar to those of the α- and β-amylases discussed above.

Amylophosphatase. Mayer (133) summarized the evidence which led to the postulation of this enzyme. The concept prevailed that phosphorus was intrinsically a part of the starch molecule. Upon enzymic liquefaction of starch a release of phosphorus was detected. This liquefaction apparently took place without the production in detectable quantities of either dextrins or reducing sugars. The name "amylophosphatase" was coined and the enzyme described as a "starch liquefier." The similarity between this enzyme and α-amylase, both in properties and mode of action, strongly suggests that the reaction observed was that of α-amylase with incidental liberation of phosphorus. Myrbäck (25) also emphasizes the fact that such an enzyme has no significance in the customarily observed amylolytic liquefaction of starch.

Phosphorylase. The researches of Cori and Cori (134) have resulted in the clear definition of a reversible enzymic system in animal tissues: glycogen + inorganic phosphate ⇌ glucose-1-phosphate. The enzyme catalyzing this reaction is known as phosphorylase. Hanes (135-136) demonstrated the presence of a similar enzyme in pea seeds and potato tubers. Unlike the apparently irreversible amylolytic splitting of starch, the action of phosphorylase is reversible. Depending on the conditions, the same enzyme either splits starch into the hexose phosphate or synthesizes a starch-like carbohydrate from hexose-1-phosphate. The phosphorylase enzyme system has been further elucidated by the Coris and their coworkers (137-140) and current concepts have been discussed in detail by Hestrin (141). The data of Bourne *et al.* (142) for potato "Q-enzyme" (143, 144) indicate that not only does this enzyme synthesize the amylopectin fraction of starch but that it may also have an amlolytic function and can hydrolyze starch. Considerable research may be required before a complete understanding of the physiological function of these various carbohydrases, both synthetic and hydrolytic, is completely understood.

Amylocytase. The existence of an enzyme which hydrolyzes the supposedly very resistant outer covering of native starch granules as a preliminary action to the degradation of the internal starch was early postulated (145). The "raw starch amylase" of Blish *et al.* (146) may well be placed in this group. This was proposed as a factor present in malt and active in the breakdown of native wheat starch granules. Further evidence (42) indicates that, at least in malts, the action cannot be separated from that of α-amylase.

Glucosidases. It is becoming increasingly evident that the majority of the amylase preparations which are available for industrial utilization contain, in addition to amylases, other carbohydrases of varied nature. These appear to have very little influence on the liquefaction and initial dextrinization and saccharification of starch but may have a marked influence on the latter stages during a prolonged hydrolysis.

Schwimmer (147) has postulated that maltase has a role in the degradation of native starches and Corman and Langlykke (15) have demonstrated an important role in starch saccharification for a mold "glucogenic" system that may be evaluated by its hydrolysis of maltose. The "dextrinase" or "limit-dextrinase" of malt described by Kneen (19) and Kneen and Spoerl (13) and the "limit-dextrinase" of fungi described by Back *et al.* (12) cannot be classified as either amylase or maltase and appear to function primarily in the splitting of low molecular weight dextrins, perhaps by cleavage of anomalous linkages.

All of these findings, although at times appearing to confuse the amylase picture, contribute eventually to a clarification of our knowledge of the amylases. Undoubtedly further research will provide additional information bearing on the properties and modes of action of this important group of enzymes. It seems a foregone conclusion that this research will also provide a more solid foundation for the industrial production and utilization of the amylases and their complimentary enzymes.

BIBLIOGRAPHY

1. Walton, R. P., A Comprehensive Survey of Starch Chemistry, New York, Part 2, 15 and 243 (1928).
2. Caldwell, M. L., and Adams, M., Enzymes and Their Role in Wheat Technology, J. A. Anderson, Editor, New York, 23 (1946).
3. Kneen, E., and Sandstedt, R. M., Enzymes and Their Role in Wheat Technology, J. A. Anderson, Editor, New York, 89 (1946).
4. Geddes, W. F., *Advances in Enzymol.,* **6**, 415 1946).
5. Lazar, O., *J. S. African Chem. Inst.,* **21**, 23 (1938).
6. Kirchhoff, M., *J. Pharm.,* **2**, 250 (1816).
7. Payen, A., and Persoz, J., *Ann. chim. phys.* (ser. 2), **53**, 73 (1833).
8. Atkinson, R. W., *Proc. Roy. Soc. London,* **32**, 299 (1881).
9. Märcker, M., *Landw. Vers. Sta.,* **23**, 69 (1879).
10. Kneen, E., *Cereal Chem.,* **22**, 112 (1945).
11. Bourne, E. J., Macey, A., and Peat, S., *J. Chem. Soc.,* **1945**, 882.
12. Back, T. M., Stark, W. H., and Scalf, R. E., *Anal. Chem.* **20**, 56 (1948).
13. Kneen, E., and Spoerl, J. M., *Am. Soc. Brewing Chemists, Proc.* **1948**, 20.
14. Witt, P. R., Jr., and Ohle, R. L., *Am. Soc. Brewing Chemists, Proc.* **1948**, 28.
15. Corman, J., and Langlykke, A. F., *Cereal Chem.* **25**, 190 (1948).
16. Fodor, A., *Ergeb. Enzymforsch,* **1**, 39 (1932).
17. Moelwyn-Hughes, E. A., *Ergeb. Enzymforsch.,* **2**, 1 (1933).
18. Moelwyn-Hughes, E. A., *Ergeb. Enzymforsch.,* **6**, 23 (1937).
19. Stearn, A. E., *Ergeb. Enzymforsch.,* **7**, 1 (1938).
20. Van Slyke, D. D., Advances in Enzymol., **2**, 33 (1942).
21. Kjeldahl, M. J., *Compt. rend. trav. lab. Carlsberg,* **2**, (1879); reprinted in *Z. ge* *Brauw., N.S.,* **3**, 49 (1880).
22. Lintner, C. J., *J. prakt. Chem., N.S.,* **34**, 378 (1886).
23. Roberts, W., *Proc. Roy. Soc. London,* **32**, 145 (1881).
24. Wohlgemuth, J., *Biochem. Z.,* **9**, 1 (1908).
25. Myrbäck, K., *Advances in Carbohydrate Chem.,* **3**, 252 (1948).
26. Hopkins, R. H., *Advances in Enzymol.,* **6**, 389 (1946).
27. Jozsa, S., and Johnston, W. R., *Ind. Eng. Chem., Anal. Ed.,* **7**, 143 (1935).
28. Landis, Q., and Redfern, S., *Cereal Chem.,* **24**, 157 (1947).
29. Jozsa, S., and Gore, H. C., *Ind. Eng. Chem., Anal. Ed.,* **2**, 26 (1930).
30. Blom, J., and Bak, A., *Z. physiol. Chem.,* **256**, 197 (1938).
31. Tilden, E. B., and Hudson, C. S., *J. Bact.,* **43**, 527 (1942).
32. McClenahan, W. S., Tilden, E. B., and Hudson, C. S., *J. Am. Chem. Soc.,* **64**, 2139 (1942).
33. Kerr, R. W., *J. Am. Chem. Soc.,* **64**, 3044 (1942).

34. Kerr, R. W., *J. Am. Chem. Soc.,* **65**, 188 (1943).
35. Sandstedt, R. M., Kneen, E., and Blish, M. J., *Cereal Chem.,* **16**, 712 (1939).
36. Olson, W. J., Evans, R., and Dickson, A. D., *Cereal Chem.,* **21**, 533 (1944).
37. Olson, W. J., Evans, R., and Dickson, A. D., *Cereal Chem.,* **24**, 299 (1947).
38. Redfern, S., *Cereal Chem.,* **24**, 259 (1947).
39. Ehrnst, L. E., and Lucht, O. C., *Am. Soc. Brewing Chemists, Proc.,* **1947**, 19.
40. Graesser, F. R., and Dax, P. J., *Wallerstein Lab. Commun.,* **9**, 43 (1946).
41. Preece, I. A., *J. Inst. Brewing,* **54**, 141 (1948).
42. Kneen, E., Beckord, O. C., and Sandstedt, R. M., *Cereal Chem.,* **18**, 741 (1941).
43. Blom, J., Bak, A., and Braae, B., *Z physiol. Chem.,* **250**, 104 (1937).
44. Hanes, C. S., and Cattle, M., *Proc. Roy. Soc. London,* **B125**, 387 (1938).
45. Caldwell, M. L., and Hildebrand, F. C., *J. Biol. Chem.,* **111**, 411 (1935).
46. Blom, J., and Rosted, C. O., *Acta Chem. Scand.,* **1**, 233 (1947).
47. Gates, R. L., and Kneen, E., *Cereal Chem.,* **26**, 228 (1949).
48. Somogyi, M., *J. Biol. Chem.,* **119**, 741 (1937).
49. Beckord, L. D., Kneen, E., and Lewis, K. H., *Ind. Eng. Chem. Ind. Ed.,* **37**, 692 (1945).
50. Kneen, E., and Beckord, L. D., *Arch. Biochem.,* **10**, 41 (1946).
51. Cleveland, F. C., and Kerr, R. W., *Cereal Chem.,* **25**, 133 (1948).
52. Swanson, M. A., *J. Biol. Chem,* **172**, 805 (1948).
53. Bernfeld, P., and Studer-Pécha, H., *Helv. Chim. Acta,* **30**, 1895 (1947).
54. Bernfeld, P., and Studer-Pécha, H., *Helv. Chim. Acta,* **30**, 1904 (1947).
55. Bernfeld, P., and Fuld, M., *Helv. Chim. Acta,* **31**, 1423 (1948).
56. Alfin, R. B., and Caldwell, M. L., *J. Am. Chem. Soc.* **70**, 2534 (1948).
57. Kerr, R. W., and Schink, N. F., *Ind. Eng. Chem., Ind. Ed.,* **33**, 1418 (1941).
58. Kerr, R. W., Meisel, H., and Schink, N. F., *Ind. Eng. Chem., Ind. Ed.,* **34**, 1232 (1942).
59. Kneen, E., and Sandstedt, R. M., *Cereal Chem.,* **18**, 237 (1941).
60. Osborne, T. B., *J. Am. Chem. Soc.,* **17**, 587 (1895).
61. Osborne, T. B., and Campbell, G. F., *J. Am. Chem. Soc.,* **18**, 536 (1896).
62. Caldwell, M. L., Booher, L. E., and Sherman, H. C., *Science,* **74**, 37 (1931).
63. Meyer, K. H., Fischer, E. H., and Bernfeld, P., *Helv. Chim. Acta,* **30**, 64 (1947).
64. Meyer, K. H., Fischer, E. H., and Bernfeld, P., *Arch. Biochem.,* **14**, 149 (1947).
65. Meyer, K. H., Fischer, E. H., Bernfeld, P., and Straub, A., *Experientia,* **3**, 455 (1947).
66. Meyer, K. H., Fuld, M., and Bernfeld, P., *Experientia,* **3**, 411 (1947).
67. Meyer, K. H., Fischer, E. H., Bernfeld, P., and Duckert, F., *Arch. Biochem.,* **18**, 203 (1948).
68. Balls, A. K., Thompson, R. R., and Walden, M. K., *J. Biol. Chem.,* **163**, 571 (1946).
69. Balls, A. K., Walden, M. K., and Thompson, R. R., *J. Biol. Chem.,* **173**, 9 (1948).
70. Schwimmer, S., and Balls, A. K., *J. Biol. Chem.,* **176**, 456 (1948).
71. Danielsson, C. E., *Nature,* **160**, 899 (1947).
72. Danielsson, C. E., *Nature,* **162**, 525 (1948).
73. Hollenbeck, C. M., and Blish, M. J., *Cereal Chem.,* **18**, 754 (1941).
74. Di Carlo, F. J., and Redfern, S., *Arch. Biochem.,* **15**, 343 (1947).
75. Kneen, E., Sandstedt, R. M., and Hollenbeck, C. M., *Cereal Chem.,* **20**, 399 (1943).
76. Chrzaszcz, T., and Janicki, J., *Biochem. Z.,* **260**, 354 (1933).
77. Chrzaszcz, T., and Janicki, J., *Biochem. J.,* **28**, 296 (1934).
78. Kneen, E., and Sandstedt, R. M., *J. Am. Chem. Soc.,* **65**, 1247 (1943).
79. Kneen, E., and Sandstedt, R. M., *Arch. Biochem.,* **9**, 235 (1946).

80. Miller, B. S., and Kneen, E., *Arch. Biochem.*, **15**, 251 (1947).
81. Militzer, W., Ikeda, C., and Kneen, E., *Arch. Biochem.*, **9**, 309 (1946).
82. Militzer, W., Ikeda, C., and Kneen, E., *Arch. Biochem.*, **9**, 321 (1946).
83. Eyster, H. C., *Plant Physiol.*, **21**, 68 (1946).
84. Di Carlo, F. J., and Redfern, S., *Arch. Biochem.*, **17**, 1 (1948).
85. Wijsman, H. P., Jr., *Rec. trav. chim.*, **9**, 1 (1890).
86. Kuhn, R., *Ann.*, **443**, 1 (1925).
87. Ohlsson, E., *Compt. rend. trav. lab. Carlsberg*, **16**, 1 (1926).
88. Ohlsson, E., *Z. physiol. Chem.*, **189**, 17 (1930).
89. Hanes, C. S., *New Phytologist*, **36**, 101, 189 (1937).
90. Weill, C. E., and Caldwell, M. L., *J. Am. Chem. Soc.*, **67**, 212 (1945).
91. Weill, C. E., and Caldwell, M. L., *J. Am. Chem. Soc.*, **67**, 214 (1945).
92. Kneen, E., *Cereal Chem.*, **21**, 304 (1944).
93. Kneen, E., *Cereal Chem.*, **22**, 114 (1945).
94. Norris, R. V., and Viswanath, B., *Agr. J. India*, **18**, 362 (1923).
95. Newton, J. M., and Naylor, N. M., *Cereal Chem.*, **16**, 71 (1939).
96. Newton, J. M., Hixon, R. M., and Naylor, N. M., *Cereal Chem.*, **20**, 23 (1943)
97. Gore, H. C., *J. Biol. Chem.*, **44**, 19 (1920).
98. Giri, K. V., *J. Indian Chem. Soc.*, **11**, 339 (1934).
99. Giri, K. V., *J. Indian Chem. Soc.*, **15**, 249 (1938).
100. Le Mense, E. H., Corman, J., Van Lanen, J. M., and Langlykke, A. F., *J. Bact.*, **54**, 149 (1947).
101. Hopkins, R. H., and Kulka, D., *J. Inst. Brewing*, **48**, 170 (1942).
102. Di Carlo, F. J., and Redfern, S., *Arch, Biochem.*, **15**, 333 (1947).
103. Effront, J., *Compt. rend.*, **174**, 18 (1922).
104. Hockenhull, D. J. D., and Herbert, D., *Biochem. J.*, **39**, 102 (1945).
105. Rose, D., *Arch. Biochem.*, **16**, 349 (1948).
106. Bois, E., and Savary, J., *Can. J. Research*, **B23**, 208 (1945).
107. Myrbäck, K., and Willstaedt, E., *Acta Chem. Scand.*, **3**, 91 (1949).
108. Little, J. E., and Caldwell, M. L., *J. Biol. Chem.*, **142**, 585 (1942).
109. Little, J. E., and Caldwell, M. L., *J. Biol. Chem.*, **147**, 229 (1943).
110. Meyer, K. H., Fischer, E. H., Staub, A., and Bernfeld, P., *Helv. Chim. Acta*, **31**, 2158 (1948).
111. Bernfeld, P., Staub, A., and Fischer, E. H., *Helv. Chim. Acta*, **31**, 2165 (1948).
112. Hopkins, R. H., and Krause, C. B., Biochemistry Applied to Malting and Brewing, New York, 1937.
113. Leberle, H., Die Bierbrauerei. I. Die Technologie der Malzbereitung, Stuttgart, 1938.
114. De Clerck, J., Cours de Brasserie. Vol. I, Louvain, 1948.
115. Takamine, J., U. S. Patent 525,823 (Sept. 11, 1894).
116. Takamine, J., *J. Indian Eng. Chem.*, **6**, 824 (1914).
117. Oshima, K., and Church, M. B., *Ind. Eng. Chem.*, **15**, 67 (1923).
118. Harada, T., *Ind. Eng. Chem., Ind. Ed.*, **23**, 1424 (1931).
119. Underkofler, L. A., Fulmer, E. I., and Schoene, L., *Ind. Eng. Chem., Ind. Ed.*, **31**, 734 (1939).
120. Underkofler, L. A., Severson, G. M., Goering, K. J., and Christensen, L. M., *Cereal Chem.*, **24**, 1 (1947).
121. Hao, L. C., Fulmer, E. I., and Underkofler, L. A., *Ind. Eng. Chem.*, **35**, 814 (1943).
122. Gates, R. L., and Kneen, E., *Cereal Chem.*, **25**, 1 (1948).

123. Adams, S. L., Balankura, B., Andreasen, A. A., and Stark, W. H., *Ind. Eng. Chem.*, **39**, 1615 (1947).
124. Erb, N. M., Wisthoff, R. T., and Jacobs, W. L., *J. Bact.* **55**, 813 (1948).
125. Le Mense, E. H., Sohns, W. E., Corman, J., Blom, R. H., Van Lanen, J. M., and Langlykke, A. F., *Ind. Eng. Chem.*, **41**, 100 (1949).
126. Effront, J., *Compt. rend.*, **164**, 415 (1917).
127. Boidin, A., and Effront, J., U. S. Patent 1,227,374 (May 22, 1917).
128. Boidin, A., and Effront, J., U. S. Patent 1,227,525 (May 22, 1917).
129. Schultz, A., Atkin, L., and Frey, C. N., U. S. Patent 2,159,678 (May 23, 1939).
130. Wallerstein, L., *Ind. Eng. Chem., Ind. Ed.*, **31**, 1218 (1939).
131. Beckord, L. D., Peltier, G. L., and Kneen, E., *Ind. Eng. Chem., Ind. Ed.*, **38**, 232 (1946).
132. Peltier, G. L., and Beckord, L. D., *J. Bact.*, **50**, 711 (1945).
133. Mayer, K., *Z. physiol. Chem.*, **262**, 29 (1939).
134. Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, **135**, 733 (1940).
135. Hanes, C. S., *Proc. Roy. Soc. London,* **B128**, 421 (1940).
136. Hanes, C. S., *Proc. Roy. Soc. London,* **B129**, 174 (1940).
137. Green, A. A., and Cori, G. T., *J. Biol. Chem.*, **151**, 21 (1943).
138. Cori, G. T., and Green, A. A., *J. Biol. Chem.*, **151**, 31 (1943).
139. Cori, C. F., Cori, G. T., and Green, A. A., *J. Biol. Chem.*, **151**, 39 (1943).
140. Cori, G. T., and Cori, C. F., *J. Biol. Chem.*, **151**, 57 (1943).
141. Hestrin, S., *Brewers Digest,* **23**, 1 (1948).
142. Bourne, E. J., Macey, A., and Peat, S., *J. Chem. Soc.*, **1945**, 882.
143. Haworth, W., Peat, S., and Bourne, E., *Nature,* **154**, 236 (1944).
144. Bourne, E., and Peat, S., *J. Chem. Soc.*, **1945**, 877.
145. Brown, H. T., and Morris, G. H., *J. Chem. Soc. (Trans.)*, **57**, 458 (1890).
146. Blish, M. J., Sandstedt, R. M., and Mecham, D. K., *Cereal Chem.*, **14**, 605 (1937).
147. Schwimmer, S., *J. Biol. Chem.*, **161**, 219 (1945).

CHAPTER XVI

MODIFICATION OF STARCH BY ENZYMES

By G. M. Severson

1. **Introduction.** Enzymes are catalysts of organic origin and play the dominant rôle in almost innumerable chemical transformations. One group promotes scission of the starch molecule or of molecular starch aggregates; the enzymes accomplishing this action are designated as amylases. This chapter is concerned with the industrial application of these enzymes for inducing a modification or change in starch. The preparation and properties of amylases as well as individual applications of the several starch modifications are reviewed elsewhere in this text.

A number of physical and chemical conditions have a marked influence on the activity of these enzymes. Thus, pH, temperature, composition of the substrate, etc., all exert effects on the reaction rate or mode of reaction. For each set of conditions, there is an interrelationship between the various factors. Certain generalizations in respect to favorable or unfavorable conditions will be discussed, and pertinent specifications will be given for each of the reactions considered.

The common sources of commercial amylase or "diastatic" preparations are the cereal malts, certain species of molds or bacteria, and, to a limited extent, animal sources such as pancreatic preparations. These are discussed in Chapter XV. The enzyme products may be subjected to some purification and concentration or made with little or no treatment subsequent to cultivation, as in the case of barley malt.

It is not within the scope of this chapter to discuss the different views on enzyme classification or mechanism. On the premise that a given enzyme is restricted in its activity to inducing a specific reaction, many commercial preparations have a very complex enzyme system; others are relatively pure in one enzyme and sold for a specific type of reaction. In any case, the user must fix the conditions to favor the desired starch change.

Starch-modifying enzymes may be grouped according to the reaction they promote, as follows: (1) liquefy or solubilize starch without inducing extensive degradation of the starch molecule ("amyloclastic" enzymes), (2) produce degradation products classified as dextrins with only small amounts of sugars ("dextrinogenic" amylases), (3) hydrolyze the starch to a high percentage of reducing sugars such as maltose or dextrose ("saccharogenic" amylases), (4) cleave the starch molecule by introduction of chemical groupings other than those from water (an example of which is phosphorylase), (5) cleave the starch molecule with a possible rearrangement of the units formed (an example of which is the action of *Bacillus macerans* amylase which forms the Schardinger dextrins).

The last two groups of enzymes have not as yet attracted the attention of industry, and therefore this discussion will be divided into the three subdivisions (1) liquefaction, (2) dextrinization, and (3) saccharification.

2. Liquefaction of Starch.

a. Liquefaction in the Starch Trade by Enzyme Action. Liquefaction of starch is understood to mean a disorganization of the starch granule into smaller physical units that remain more permanently dispersed than gelatinized, untreated starch. The most significant visible effect is a reduction in the viscosity or thickness of the final paste, and this should be attended by a minimum production of reducing substances such as lower dextrins and sugars.

A number of investigators believe that a definite enzyme is responsible for starch liquefaction and that selected linkages are attacked. Hanes (1) gives an interesting picture, reasoning that molecular starch chains are terminally bound by esterified phosphoric acid and that the liquefying enzyme specifically attacks this linkage. Other workers believe that the enzyme responsible for liquefaction likewise produces dextrins as commonly defined. A number of investigators (2, 3) show a parallelism between liquefaction and dextrinization under varying conditions and reason that one enzyme is responsible for both dextrinization and liquefaction.

At any rate, the enzyme component responsible for starch liquefaction is commonly referred to as α-amylase, and many excellent commercial preparations of this enzyme are available. Indeed, enzyme manufacturers seem to have concentrated their efforts in recent years on the perfection of enzymes for this purpose. This fact may reflect the growing importance of this type of starch modification in industry. Among those which might be listed as for sale in America are Amyliq, Arcy, Rhozyme-DX, Vanzyme, and many others possessing high efficiency.

In practice, liquefactions of corn starch are performed as follows: The starch is made up in a slurry with cold water at some convenient concentration (usually 10 parts of water to from 1 to 2 parts of starch), the enzyme is mixed in, and agitation and heat applied. About 0.1 to 1.0% of enzyme is required (based on the weight of starch treated), the amount depending on the extent of liquefaction desired and the operating conditions used. It is also desirable to adjust the pH of the conversion mixture, when necessary, to the optimal range recommended by the enzyme manufacturer, which, for the type of liquefaction described and the conditions outlined, varies from pH 5.5 to 7.0. Live steam, blown through jets into the bottom of the conversion tank, is commonly used for heating. This also supplies considerable agitation.

Common practice is to heat the conversion slurry up to a temperature in the gelatinization range for the starch as quickly as possible and then to maintain this temperature until the desired reaction stage has been reached. Finally, the mixture is heated to an elevated temperature to complete the conversion, to disperse the converted starch thoroughly and finally to inactivate the enzyme. This enzyme inactivation prevents unwanted action in subsequent processing. For corn starch, conversion temperatures vary from about 160°F. for the more thermolabile enzyme preparations up to 180°F. for those of greater thermostability. A conversion time of about 30 minutes is often used and the process is completed at a temperature of 200-205°F.

Some operators prefer to heat the starch slurry slowly up to the inactivating temperature. Others claim that greater enzyme economy results from the addition of the enzyme in small amounts at various stages of the heating. However, few, if any, attempt to secure the maximal enzyme economy by first gelatinizing the starch and then cooling it to a favorable temperature for enzymic activity.

Starch-splitting enzymes act with great difficulty on ungelatinized starch, although a specific enzyme has been postulated for the action observed on native wheat starch (4). Kerr *et al.* (5) noted the action of malt on ungelatinized corn starch. However, the organization of the starch granule is such that liquefaction does not take place to any appreciable extent until the granule structure is weakened by gelatinization. As mentioned above, enzymes are inactivated by heat; so the two effects of the heat must be properly balanced; *i.e.,* gelatinization of the starch and enzyme destruction. Hence it may be found that a factor found optimal for the enzyme under certain conditions must be changed, in order to obtain the best results when applied under other circumstances. For example, one of the most important factors influencing converting conditions is the pH value. Under certain favorable conditions, such as the use of gelatinized starch at 120°F., many amyloclastic enzymes show a peak activity at pH 5.0 to 5.5. At higher temperatures, this pH becomes increasingly destructive to the enzyme. At pH 6.5, the enzyme is more tolerant toward heat. Therefore, a higher pH range is used for actual plant operation than is indicated as optimum in published data, since a relatively high temperature is needed for corn starch gelatinization.

This point of the interrelationship of the various factors influencing enzymic activity constantly must be kept in mind when enzymes are used for starch degradation. There are no such values as optimal pH, optimal temperature, etc., except for one particular set of conditions, and with a particular starch.

These general rules may be set up.

a. The rate of enzyme destruction increases with temperature.

b. The higher the temperature, the faster the rate of action becomes for a given enzyme, up to the point at which enzyme destruction overbalances this effect.

c. The higher the pH (up to and not exceeding 7.0), the more stable is α-amylase toward heat.

d. The stabilities of the amylases are dependent to some extent on certain subsidiary factors; an example of this is the stabilizing action of the calcium ion on α-amylase.

e. For the complex amylase systems, as found in barley malt, the temperature influences the mode of reaction; *e.g.*, when barley malt is used at 150°F., sugar formation will be considerably less than if the reaction proceeds at 135°F. This is due to the difference in thermostability of the α and β components.

f. Under comparable conditions the order of thermostability of the *α-amylase* types is bacterial > malt > fungal.

g. For effective enzyme action, starch must be gelatinized or otherwise made accessible to the action. If the enzyme action proceeds during the course of gelatinization, it would thus follow that a starch which gelatinizes and becomes accessible to the enzyme at lower temperatures permits use of an enzyme of less thermostability.

Enzyme-converted starch is prepared as a fluid product. The resulting paste or sol costs more to dehydrate to a dry product than ungelatinized starch. To transport this modified product without dehydration from starch-manufacturing plants to its points of use, as for example in textile and paper mills, would add more to the basic cost of the starch than these industries can afford to pay. Hence this type of starch modification is usually performed at the point of use. This arrangement has worked out very well. The starch and enzyme chemists from their respective industries have worked with the customer until today many large paper and textile mills and related industries perform this modification of starch in a relatively competent fashion.

Starch manufacturers have on the market special products designed for these uses. The prime requisites in a starch or enzyme conversion are (1) that the modified starch product should be adaptable for the desired use and (2) that conversion should proceed rapidly with a minimum amount of added enzyme.

Judging from the literature, little is known concerning the improvement of starch to condition it for special use after the enzyme conversion and such modification may be difficult even though the required characteristics are known. For example, if an enzyme-converted starch is to be used as an adjunct in the clay coating of paper, the starch product should be of low viscosity, exhibit a minimum of plastic effects, and yet possess great strength in binding clay to paper fiber. As outlined by Kerr (6), these characteristics are to be found in the fractions of starch which show the highest degree of colloidal stability. This applies particularly to corn starch, since the so called linear molecules of corn starch are unstable; that is, these molecules tend to retrograde and possess low adhesive strength. As retrogradation proceeds in a clay coating paste, viscosity and plasticity are increased, and the uniform application of the clay coating becomes more difficult. The above discussion is given to show that frequently it is a fraction of starch rather than the whole starch in the liquefied state which is desired. Although effective methods are known for fractionating starch (7-9), at present the processes are relatively expensive and their use would raise the cost to or above the price of competitive products, which in the above example could be casein. However, Kerr and coworkers (5) outline a reasonably economical procedure by which it is claimed that enzyme treatment removes, partially at least, the linear fraction in preference to the branched colloidally stable fraction.

For improvement in the ease of starch liquefaction, three approaches may be used: (1) addition of substances which promote enzyme action, often referred to as enzyme accelerators or stabilizers, (2) removal of enzyme-toxic materials, and (3) partial modification of the starch prior to the enzyme action. It must be kept in mind that any practical treatment as listed above must be of less cost in proportion to the advantage obtained than that obtained by adding a little extra enzyme to the original starch.

The literature abounds in references to so called enzyme accelerators. These substances which promote enzymic action may be grouped under four different headings which by their action (1) act as true accelerators, (2) exert solubilizing or peptizing actions on the enzyme, (3) stabilize the enzyme toward the conditions imposed, and (4) create more favorable conditions for enzymic action. Because of the vagueness of definition, the first action listed is probably more for theoretical discussion than of industrial significance. Solubilization or peptization is usually not important when industrial enzyme preparations are used under usual pasting conditions. The marked accelerating action of some of the neutral salts on pancreatic amylase might be explained on this basis. A number of compounds, both organic and inorganic, are said to stabilize enzymes, and while many of these are of doubtful significance, the calcium ion is an example of one which has found industrial application. The tolerance of α-amylase toward heat under usual conditions of production of starch pastes is definitely increased by the addition of small amounts of calcium* (10, 11). The most significant and perhaps only pertinent group of materials to be considered under the heading of improving conditions for enzymic activity are those substances which adjust the pH to a level more favorable for enzyme action. Thus starches to be used for enzyme conversion are usually adjusted to approximately pH 6. Inasmuch as water varies considerably in hydrogen ion concentration and because different enzyme preparations have different ranges of pH for optimal activity, the user should check the pH of his conversion mixture against the data for pH optima supplied by the manufacturer of the enzyme used.

Likewise numerous inhibitors also may be listed. The copper ion is the common one which in trace amounts can seriously retard α-amylase action.

Certain methods of weakening the granule structure, which presumably make access easier for the enzyme at lower temperatures, result in a starch which will liquefy to a given level with greater ease. These methods include dry heat treatment of starch, ball milling, and acid treatment under certain conditions.

b. Liquefaction of Cereal Mashes for Fermentation. The use of amylase in making up the mash prior to cooking is a common practice in the manufacture of alcohol from cereals. The ground grain and water are mixed and the liquefying agent added, usually prior to reaching the temperature of starch gelatinization. Preliminary thinning of the mash enables the operator to cook a more concentrated mash and results in savings in steam and in the costs of equipment. It also may render the mash more accessible to enzyme action in saccharification after cooking.

A satisfactory and applicable procedure is as follows: Mix the ground grain with water containing a suitable liquefying enzyme in proper concentration, in the ratio of 1 part of grain with 2 parts of water at a temperature just under that at which starch granules start to swell, and maintain it for 10 to 15 minutes to allow penetration into the grain particles. The amylase must be one having good activity throughout the temperature range in which the starch gelatinization occurs. Raise the temperature of the slurry during 15 to 20 minutes through the gelatinization range to 180-185°F., which is above the point of maximum viscosity, and then proceed with the cooking operations.

---

*The preferential stabilizing and apparent accelerating effect of the alkaline earths, particularly calcium, on the α-amylase component of diastases and the apparent retarding effect on the β-amylase component, as well as the apparent increase in the pH for optimal activity of the amylases with an increase in the temperature of reaction have been disclosed by Kerr and Trubell in U. S. Patent App. 279,274 (1939) and in the French Patent 863,043 (1940).

Corn is a common cereal used for the manufacture of industrial alcohol. Since corn has a wide gelatinization range (between 145 and 180°F.), the enzyme employed must be relatively thermostable for maximum efficiency. For this range, a bacterial preparation is superior to malt. If wheat is used as the raw material for alcohol manufacture, barley malt is very effective inasmuch as the apparent gelatinization range for wheat starch occurs at a lower temperature than for corn starch. The relatively thermolabile fungal preparations are unsatisfactory in either case.

c. Desizing. The object of desizing starch-sized fabrics is to solubilize the starch so that it may be removed by washing the fabrics. All three types of action, however, may take place; that is, liquefaction, dextrinization, and saccharification. Desizing is listed under liquefaction because both liquefaction and desizing are thought of as solubilizing actions. Cereal, bacterial, or fungal types of enzyme preparations are used.

There are many modifications used in desizing operations, and each factory has specific conditions which dictate the exact method to be employed. In essence, the cloth is treated with the enzyme, the action is allowed to take place, after which the fabric is washed. The process may be continuous or may be carried out on batches of material. A consideration of two procedures in which the enzyme action takes place will show the variance in conditions used. After application of the enzyme, the treated cloth may be pressed to remove excess enzyme and then run into a warm chamber in which the enzyme action proceeds for 30 to 60 minutes, or it may simply be stacked overnight, before it is washed. Gale (12) presented a discussion of desizing and also included enzymic treatments used in laundering and cleaning.

3. **Dextrinization.** Dextrins are degradation products of starch. During the course of shortening or cleaving the starch molecule, whether by enzymic or acid treatment, there is a gradual change in certain properties. The reducing power and the solubility increase, while the viscosity of the paste solution decreases, and starch originally staining blue with iodine gradually loses this property. The name dextrin is assigned to the class of compounds which fall in the zone in which these properties no longer can be considered typical of starch yet before the degradation has resulted in the formation of sugars.

a. Dextrins for Adhesives. Dextrins are used extensively in the preparation of adhesives. The preparation from the raw starches is similar to that for thin boiling starch, except that starch degradation proceeds further. Enzymes from cereal, bacterial, or fungal sources are used, although the fungal ones are not adaptable for use in dextrin formation from native corn or other starches having a relatively high temperature range for gelatinization. Corn, potato, and tapioca starches are all used, depending on the special use for the resultant paste. For malt and corn starch, a conversion temperature of 150-160°F. is generally used. This temperature is quite favorable to the dextrinizing enzyme and prevents extensive sugar formation by the saccharifying component. Instead of maintenance at this temperature, heating may be continued throughout the gelatinization range. The entire amount of enzyme may either be added at once at 150°F. or as small additions throughout conversion. Higher temperatures become applicable when bacterial enzymes are used, and of course lower temperatures must be employed for fungal amylases. The enzyme usually is inactivated by further heat treatment when the desired fluidity is reached.

The dextrin paste may be dehydrated to a powder by spray-drying. Paste makers often evaporate the paste to the desired consistency and sell it as such. They also, on occasion, blend in certain amounts of torrefaction dextrins, small amounts of borax, and a plasticizer such as cyclohexanol or glycerol or just a plasticizer for making library pastes, etc.

4. Saccharification. Saccharification involves the formation of simple sugars such as maltose or glucose from starch or other high sugar polymers. This process is, of course, basic to the fermentation industry and has also become an important adjunct to the starch trade. Although two divisions are made in this section there are other important applications. For example, saccharification is vital for bread-making when flour is not supplemented with sugar for yeast food. In fact, the rôle of amylases in baking is quite complex and not confined merely to saccharification (13).

a. Saccharification for Alcohol Production. Yeast can utilize only simple sugars. Therefore starchy materials must be saccharified before conversion to ethyl alcohol. In the manufacture of alcoholic beverages, alcohol formation is not the only important consideration, and the type and amount of saccharifying agent employed is dictated not only by the amount and conditions necessary for sugar formation but by the imparted flavors, aroma, etc. Thus, from 75 to 100% barley malt is employed in brewers' mash, and it is obvious that the amount of saccharifying enzymes present is in excess of the quantity necessary in a method involving the efficient saccharification of the starch present. Individual enzyme components can produce sugars by their action on starch. Thus, β-amylase will convert starch to approximately 60% maltose. α-Amylase, in addition to producing dextrins, also produces reducing fermentable sugars. However, complex enzyme systems are undoubtedly responsible for commercial saccharification processes in which a relatively fast and complete breakdown to fermentable sugars is required. Thus, postulated enzymes such as α-glucosidase (5) or maltase may play a very important rôle in efficient and complete saccharification of starch. A test for enzyme potency which measures only one factor (such as the Lintner value of malts) should be regarded only as an index and not as an absolute criterion of the value of that enzyme preparation in saccharification for alcohol production. The final and true value of an enzyme product for use in this type of saccharification is best judged by data of the alcohol yield. A thorough discussion of the enzyme characteristics of different amylases is given by Eric Kneen earlier in this book. The discussion following concerns processes in which the only objective is efficient saccharification and therefore is applicable more directly to the manufacture of industrial alcohol.

Barley malt is the usual enzyme source employed by the alcohol-manufacturing trade. Numerous other enzyme preparations have been used to accomplish starch conversion, notably certain species of molds. The enzyme systems of the malted cereals and the fungal preparations are both complex but differ chiefly in the relatively large percentage of β-amylase in malt and the large amount of α-amylase with none or only traces of β-amylase in the fungal preparation. Under proper conditions, both enzyme sources give efficient saccharification, and approximately the same alcohol yields are to be realized with either.

The amount of barley malt added to the cooked mash is usually 8 to 10%, on the basis of the grain weight, and the temperature is maintained at approximately 140°F. for 12 to 60 minutes, after which the mash is cooled to the fermenting temperature. With the usual conditions, a pH of 5 is generally considered to be about optimum. It should be mentioned that cooking adequate for a thorough disruption and dispersion of starch particles is necessary for complete saccharification. Additional enzyme action proceeds during the course of the fermentation.

While fungal preparations have not been extensively used in the industrial alcohol manufacture, there are numerous references to the use of this type of amylase. Renewed interest in the application of fungal amylase (in particular, the use of *Aspergillus oryzae*) has resulted from the work of recent investigators (Fulmer, Underkofler, and others (14-16) and Beresford and Christensen (17)) who have studied optimal conditions on the basis of resultant alcohol yields from various common starchy materials.

The mold can be satisfactorily grown on wheat bran, and from 2.5 to 6% of this material is adequate for maximum alcohol yields. The use of the lower amount is satisfactory if the mash has been quickly cooled according to the method as outlined by Beresford and Christensen (17). This claim is substantiated by Underkofler (18). The enzyme may be added at 130°F. during the course of cooling the mash or at the fermentation temperature.

The optimal pH is approximately 5.0. The chief advantages of mold bran as advanced by the above investigators are the lower cost of saccharification and as good or better alcohol yields in comparison to those of barley malt.

b. Production of Enzyme-converted Sirups. Starch-hydrolyzing enzymes also are used in the preparation of sugar sirups. Distinguishing features of sirups of this type are sweetness, high fermentability of dry solids, and stability toward crystallization in spite of high sugar concentration. These effects are due, in part at least, to the total high sugar concentration of which the proportion of maltose is relatively high.

A method for making these enzyme-converted sirups from corn starch is, according to Kerr and Schink (19) as follows:

A starch suspension is made up to 12° Bé. and converted under 30 lbs. of steam pressure until the "purity" (dextrose equivalent, calculated from the reducing value) reaches about 57. The converted material is then clarified, and the acidity is adjusted to about 5.0 and the temperature to 130°F. A malt diastase preparation of high Lintner value is then added (at 250° Lintner about 0.5% enzyme, on a dry solids basis, is necessary). Conversion is continued until no further increase in the "purity" is noted. A limiting "purity" of about 64 is usually attained. The liquors are then clarified and concentrated to the desired content of solids, usually about 42° Bé. In all of the finishing operations it is necessary to control the pH and temperature carefully, so as to secure the proper sugar balance and the maximum results from clarification treatments.

Other conditions can be used (20). For example, a starch suspension of higher density can be used which is converted to a sirup with a "purity" of 50. For products with lower final values for the dextrose equivalent, it is permissible to use higher initial starch concentrations, as reversion of dextrose to gentiobiose depends on the dextrose concentration. The products of acid conversion are submitted to a clarification treatment, concentrated to 30° Bé., cooled to 135°F., adjusted to pH 6.5, and the solution is converted with a fungal type of amylase to the desired sugar content. Clarification and final concentration follow.

There are many other important applications for enzyme preparations than those outlined above. Since enzymes are more or less specific in their action and control is relatively simple, their value and worth are becoming increasingly apparent. The fundamental research to which so many workers have contributed has made possible the notable advances in this field.

BIBLIOGRAPHY

1. Hanes, C. S., *New Phytologist,* **36**, 101, 189 (1937).
2. Hollenbeck, C. M., and Blish, M. J., *Cereal Chem.,* **18**, 754 (1941).
3. Blom, J., Bak, A., and Braae, B. A., *Z. physiol. Chem.,* **250**, 104 (1937).
4. Blish, M. J., Sandstedt, R. M., and Mecham, D. K., *Cereal Chem.,* **14**, 605 (1937).
5. Kerr, R. W., Meisel, H., and Schink, N. F., *Ind. Eng. Chem.,* **34**, 1232 (1942).
6. Kerr, R. W., *Paper Trade J.* (TAPPI), **115**, 30 (1942).
7. Schoch, T. J., *Cereal Chem.,* **18**, 121 (1941).
8. Kerr, R. W., and Severson, G. M., *J. Am. Chem. Soc.,* **65**, 193 (1943).
9. Pacsu, E., and Mullen, J. W., *J. Am. Chem. Soc.,* **63**, 1168 (1941).
10. Wallerstein, M., U. S. Patent 905,029 (1909); Kerr, R. W., and Trubell, O. R., (to Corn Products Ref. Co.) French Patent 863,043 (1940).
11. Kneen, E., Sandstedt, R. M., and Hollenbeck, C. M., *Cereal Chem.,* **20**, 399 (1943).
12. Gale, R. A., *Wallerstein Laboratory Commun.,* **4**, No. 12, 112 (1941).
13. Kneen, E., and Sandstedt, R. M., *Cereal Chem.,* **19**, 181 (1942).
14. Underkofler, L. A., Fulmer, E. I., and Schoene, L., *Ind. Eng. Chem.,* **31**, 734 (1939).
15. Schoene, L., Fulmer, E. I., and Underkofler, L. A., *Ind. Eng. Chem.,* **32**, 544 (1940).
16. Hao, L. C., Fulmer, E. I., and Underkofler, L. A., *Ind. Eng. Chem.,* **35**, 814 (1943).
17. Beresford, H., and Christensen, L. M., Idaho *Expt. Sta. Bull.,* No. 2412 (1940).
18. Underkofler, L. A., *Brewers Digest,* **17**, 29 (1942).
19. Kerr, R. W., and Schink, N. F., *Ind. Eng. Chem.,* **33**, 1418 (1941).
20. Dale, J. K., and Langlois, D. P., U. S. Patent 2,201,609 (1940).

CHAPTER XVII

MISCELLANEOUS REACTIONS

Starch reacts or appears to react with several types of reagents other than those that have already been considered. At present, only one of these has any technical significance worthy of note, but it is quite possible that the application of others will be made as the fundamentals involved become more clearly understood. Of the remaining reactions that have not been discussed, those of starch with complexing agents, such as iodine and polar substances, aldehydes and other cross-linking reagents and gaseous hydrogen are the most interesting.

1. Reaction of Starch With Complexing Agents.

One of the earliest observed and one of the most characteristic reactions of starch is its ability to form an intense coloration with iodine. Most frequently the color is blue, but on occasion shades through purple to red are observed. Many theories have been proposed to explain this effect which was observed early in the development of starch chemistry when the experimental background was inadequate for anything more than a guess. Recent research has clarified the situation, materially, and has offered an intelligent and logical picture of the mechanism of the reaction.

Apparently the discovery that the addition of iodine to dilute starch solutions results in a blue coloration is attributed to de Claubry (1). In 1814, he reported that the reaction is characteristic, regardless of the source from which he obtained the starch. Subsequently it was observed by Leroy (2) that water was necessary for the reaction to take place. This conclusion was substantiated by Stocks (3) who studied the effect of iodine vapor on starch. Stocks, as well as Mylius (4), Roberts (5), Hale (6), and others of this period, believed that hydriodic acid, or an iodide, was the active reagent. Roberts supported his contention by showing that pure iodine in chloroform, free from hydriodic acid, gave no blue color when added to starch, but that if the solution was heated or if an acid was added, the blue color was formed. Harrison (7) found that certain salts aided the reaction.

Pickering (8) made the observation that the blue, starch-iodide coloration disappeared when the solution was brought to the boiling point but reappeared when the solution was cooled. The temperature at which the color became apparent again was not fixed, but varied with conditions such as the concentration of starch and iodine. These results have been confirmed by Vogel (9), Radley (10), Phol (11), and others. Phol found that the intensity of the color which appeared increased as the temperature decreased, and drew the significant conclusion that hot water has a greater affinity for starch than has iodine but that iodine has a greater affinity for starch than has cold water. Another significant conclusion, drawn by early workers, is that of Payen (12, 13), who stated that the loss in blue coloration on heating is due to the expansion of the starch aggregates. On cooling, the aggregates assume their original form and the blue color reaction can again take place.

Acids and certain salts, particularly potassium iodide and sodium sulfate (14-16), have been found to increase the sensitivity of the reaction. Alkali, chloral hydrate, and certain other substances can inhibit the reaction (17, 18).

To account for the blue color, some have assumed an adsorption phenomenon in which colloidal aggregates were involved, others have postulated a complex formation with starch molecules, while still others have assumed that a definite compound was formed. Especially during periods when starch was believed to consist of only one molecular species, has the latter theory been advocated.

Among the earlier workers who considered that a definite compound was formed were Schoenbein (19) and Rouvier (20). Rouvier reported that a series of compounds was formed according to the general formula $(C_6H_{10}O_5)_{16}I_n$, where n increased from 2 to 5 as the amount of iodine added to the starch was increased. The actual amounts of iodine found in these compounds varied from 3 to 19.6%. Toth (21), however, obtained starch iodides containing as high as 22.8% iodine by dissolving starch in hot glycerol and precipitating the compound by the addition of a solution of potassium iodide and iodine. Neither these nor other workers apparently agreed on the combining proportions between iodine and the "starch molecule." Padoa and Savarè (22) found 19% iodine in the compound, while Andrews and Goettsch (23) obtained two different compositions, one corresponding to the formula $(C_6H_{10}O_5)_{12}I$ and the other to $(C_6H_{10}O_5)_{12}I_2$. Von Euler and Myrbäck (24) found two compounds also but gave as formulae $(C_6H_{10}O_5)_{18}I_2$ and $(C_6H_{10}O_5)_{18}I_4$.

The adsorption theory gained considerable support from the work of Lottermoser (25, 26), who obtained typical adsorption curves. His measurements were based on the determination of an electric potential in the system, free iodine iodide ion, as formulated by the Nernst equation, and also on the distribution ratio of free iodine between 0.1 N KI solution and CCl_4. The electric potential measurements especially should be called to attention, since recent work based on potentiometric measurements has proved of great value in a clarification of the problem.

Other methods of physical chemistry employed in such studies have been those for determination of osmotic pressure (27) and freezing point depression (28), colorimetric methods (29), and conductivity measurements (30)

It is believed significant that Berczeller (31) found that starch adsorbed no iodine from solutions of iodine in CCl_4 and found that the amount adsorbed from alcohol was less than that from water.

Again, the heterogeneity of starch seems to have received little attention in explanations advanced for the adsorption phenomena. Hanes and Cattle (32), for example, concluded that the starch molecule contains groups along the molecular chain which can adsorb iodine to give the characteristic coloration of starch. They considered that, as hydrolysis of the molecule by β-amylase proceeds, there is a progressive decrease in the number of adsorbing groups, which decrease accounts for the resulting changes in color from blue to purple to red. Hanes (33) had proposed a helical configuration for starch molecules with a periodicy of six glucose units along the length of the helix, and pointed out as significant, that when the chain length had been reduced to less than six units, the molecule is unable to give the characteristic color when treated with iodine. Freudenberg and co-workers (34) developed the idea of a helical configuration and showed from a space model that the interior of the helix is large enough to accommodate the iodine molecule. The model showed that hydroxyl groups are on the exterior of the helix and that the interior is lined with C-H groups. They proposed that iodine enters the helix and acquires the characteristic blue color because of the hydrocarbon-like surroundings, in much the same way as iodine changes color in the transition from an aqueous solution to a non-polar solvent.

In reviewing this early work, it appears that the observation of Maquenne and Roux (35), and others, that the blue color which develops with iodine is the result of a reaction with one component of starch, called amylose, and that the other component, or components, are relatively inactive, was largely overlooked until the beginning of the last decade. However, it should be recalled that with the possible exception of fractionation by electrodecantation, which was apparently developed to a high degree of perfection for potato starch by Samec and co-workers, fractionation techniques were relatively crude and the mixtures obtained probably gave indefinite results. With the accumulation of evidence to show that the common starches contain both linear and branched molecules and the development of effective means to separate the two, it became increasingly evident that the starch-iodine reaction is dependent on the structure of starch molecules and in particular on the configuration which linear starch molecules may assume under certain conditions.

Linear starch molecules in a crystalline form which gives the so-called "V" pattern by x-ray diffraction, reacts in the dry state with iodine vapor whereas dry starch in granular form or in a retrogaded state reacts feebly, if at all.

The "V" modification results when amylose is crystallized by the addition of butanol, or certain other complexing agents, to aqueous solutions. Thus, in a study of the platelet crystals of corn amylose made by Kerr and Severson (36), Rundle and French (37) found that a high percentage by weight of iodine vapor could be absorbed and moreover the x-ray diffraction pattern of the crystals was not materially altered by such treatment. Rundle and French proposed that whereas amylose molecules assume extended chain configurations in granular starch, in solution and in retrograded form, the addition of crystallizing agents such as butanol causes these chains to assume the helical configuration, postulated by Hanes, Freudenberg and others, and that in the dry state the iodine molecules enter the space within the interior of the helices formerly occupied by the butanol molecules. The crystalline platelets appeared to be substantially uniaxial when examined under crossed nicols. On edge, they were found to be quite birefringent and using a red-plate it was found that the retardation of light with its electric vector parallel to the surface of the platelet is greater than the retardation of light with its electric vector normal to the platelet. After treatment with iodine vapor, it was observed that the platelets are light blue when viewed with light normal to the platelet surface and again appear to be uniaxial. On edge they are extremely dichroic; light with its electric vector normal to the plate is very strongly absorbed, while light with its electric vector in the plane of the platelet is very weakly absorbed. These facts were believed to show that the crystals consist of closely packed helices arranged with their axes normal to the largest surface of the platelet, and that the iodine molecules on entering the helices have their long axes normal to the plane of the platelet also, that is, parallel to axis of the helix.

Subsequently, Rundle and French (38) and Rundle and Edwards (39) studied butanol-precipitated amyloses both in the wet and dried states and by means of x-ray diffraction patterns confirmed the proposal for a helical amylose chain. The results showed a helix diameter of 13.7 A., a length per turn of about 8 A., and six glucose residues per turn, with alternate helices directed in opposite directions. The helices approximate a close-packed arrangement and confirmation was obtained from spacial relations between the starch-iodine complex and the dried butanol precipitates. In the former, the iodine molecules occupy the interior of the helices, the space previously occupied by the butanol. As high as 26% of iodine by weight was found to be absorbed, which would correspond to one iodine per helix turn of six glucose residues.

Therefore, although dry starch in some forms does not react characteristically with iodine and water appears to be necessary for the reaction to take place, the latter is not necesarily the case if the amylose is already in the proper configuration for complex formation. The conclusion follows that water, as in the gelatinization of starch to form a sol, assists the reaction in that aqueous dispersion provides the opportunity for freedom of movement of amylose molecules and iodide ion may assist in the formation of the required helical configuration from the normal, more or less extended shape.

Rundle and Baldwin (40) studied the interaction between iodine and starch molecules in solution and noted that those starches which contained amylose exhibited dichroism during flow. It was found that light with its electric vector parallel to the line of flow was more strongly absorbed than light with its electric vector normal to the flow lines. This result was shown to require that the long axes of the iodine molecules in the complex be parallel to the long axis of the starch-iodine complex. Obviously, this condition would be fulfilled if helices formed from linear molecules in solution were filled with iodine similarly to the arrangement in crystalline amylose, discussed above. Waxy starches and glycogen when treated with iodine exhibited little or no dichroism. Furthermore, the butanol-precipited fraction from both corn and potato starches exhibited a noticeable dichroism during flow, even in very dilute solution whereas the non-precipitable fractions showed a doubtful dichroism.

The iodine electrode has been used by several workers to study the mechanism of complex formation between starch and iodine in solution. As noted above, in early work Lottermoser concluded from studies on the distribution of iodine between aqueous solutions of starch and carbon tetrachloride, that the iodine was taken up by the starch according to a Freundlich adsorption isotherm. Correction was made for the formation of triiodide ion, but it was assumed that iodide ion was not adsorbed by the starch. Murray (41) repeated the work, taking into account the formation of iodide ion both by hydrolysis and by reduction of iodine by the starch, as well as the formation of triiodide ion and found definite complex formation in a ratio of two atoms of iodine for every six glucose residues.

Bates, *et al.* (42) differentiated amylose from amylopectin molecules by titration with iodine using an iodine electrode and developed an analytical method for determining the composition of starch materials. It was found that the titration of amylose occurs in three steps. The initial addition of iodine increases the activity of iodine up to a certain value, as measured by the potential of the iodine electrode against a normal calomel electrode. At this point the iodine reacts with the amylose and on further addition of iodine the activity remains fairly constant until sufficient iodine has been added to react with the amylose. The activity then rises and approaches the

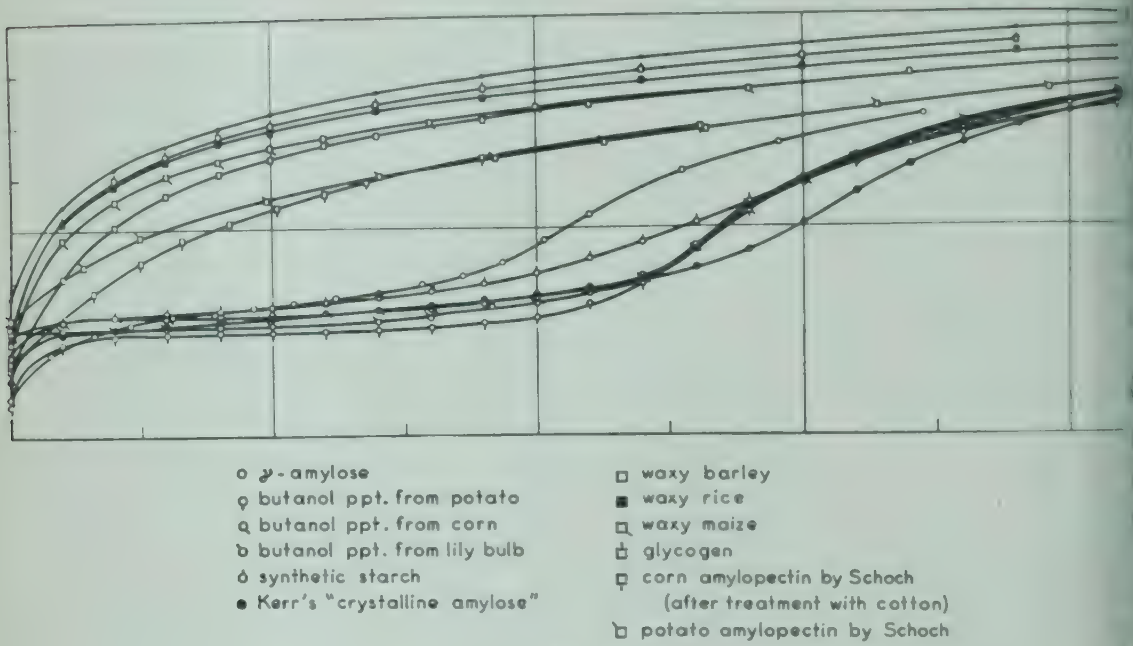


FIG. 97

value obtained if the titration were carried out in the absence of the amylose. Their results are shown in Fig. 97 from which it will also be seen that in the titration of branched starch materials, including glycogen, the iodine activity increases uniformly and rapidly to a much higher value than is obtained during the titration of amylose. The potential at the mid-point of the titration of an amylose was called the characteristic potential and this was found to increase with the length of the chain in the several amyloses. Foster (43) related this characteristic potential, E, to chain length by the equation:

$$E = A + B\sqrt{\frac{1}{n}}$$

where A and B are constants and n is the number of glucose residues in the amylose chain.

Bates and co-workers found that the amount of iodine bound by amylose varies inversely with the iodide concentration. However, maintaining the latter at 0.05 N potassium iodide, the amount of iodine bound by corn crystalline amylose was found to approach 19% by weight when the results of the titration were plotted as shown in Fig. 98. In order to obtain the weight of iodine bound by amylose at any iodine concentration, the values obtained with iodine and potassium iodide, the uppermost curve in Fig. 97, were used to calculate the concentration of free iodine corresponding to any potential and this value was subtracted from the total amount of iodine added. I

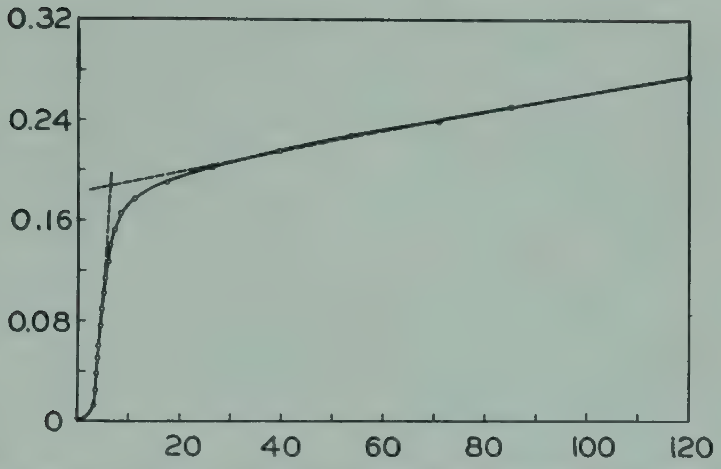


FIG. 98. Amount of iodine bound by crystalline amylose. Ordinate, g. iodine bound per g. of amylose; Abscissa, iodine concentration (moles per 10^8 liters).

will be seen from the figure that nearly all of the added iodine is bound in complex formation by the amylose until the vicinity of the inflection point is reached, which represents completion of complex formation.

More recently, Dube (44) has modified the titration procedures by using a second iodine electrode for reference in place of the calomel electrode. This arrangement is essentially a concentration cell in which the electromotive force is a function of the concentrations of iodide and iodine in one half cell and the concentrations of free iodide and iodine in the half cell used for the titration. From this study the following set of equations were proposed for the reaction between iodine, iodide ion and amylose:

$$\mathrm{Am(aq)} + \mathrm{NI_2(aq)} \rightarrow \mathrm{Am(I_2)_N(aq)}$$
$$\mathrm{Am(I_2)_N(aq)} + \mathrm{NI^-(aq)} \rightarrow \mathrm{Am(I^-_3)_N(s)}$$
$$\mathrm{Am(aq)} + \mathrm{NI^-(aq)} \rightarrow \mathrm{Am(I^-)_N(aq)}$$
$$\mathrm{Am(I^-)_N(aq)} + \mathrm{NI_2(aq)} \rightarrow \mathrm{Am(I^-_3)_N(s)}$$

wherein Am represents amylose, (aq) and (s) indicate solution and solids phases, respectively, and N represents the relative number of iodine molecules and iodide ions reacting. It was concluded that the triiodide complex is in the solid phase, as shown above, and it was found not possible to distinguish between the amount of iodine bound as I_2 and as I_3.

The pseudo-equilibrium constant per mole of iodine was calculated for the reaction:

$$\mathrm{Am(aq)} + \mathrm{NI_2} + \mathrm{NI^-} \rightarrow \mathrm{Am(I^-_3)_N(s)}.$$

The value obtained was

$$(K)^{\frac{1}{n}} = 1.0 \times 10^9$$

The heat of reaction was found to be —19,600 cal. per mole of iodine at 25°C. and the stability of the iodine in the complex is therefore comparable with that of iodine held by weak primary valence bonds.

Baldwin *et al.* (45) investigated the reaction by a quantitative study of the absorption spectra of starch components when treated with iodine. Because of its exceptional purity, corn crystalline amylose prepared by the method of Kerr and Severson was selected for study of the iodine-iodide relationships in the formation of the complex. Titrating 0.01% solutions of amylose with iodine at concentrations of iodide between 0.5 *M* to 0.001 *M* and following the formation of the iodine-amylose complex by spectrophotometric measurements, it was found that the percentage of iodine in the complex decreased as the concentration of iodide increased. When the number of glucose residues per iodine molecule in the complex was plotted as a function of iodide concentration, it was found that the former varied as the fourth root of the latter and this curve was extrapolated to a value of six glucose residues at zero iodide concentration, in excellent agreement with the ratio found by Murray.

Simerl and Browning (46) had discussed the spectrophotometry of the blue color produced by treating starch and its components with iodine, which subsequently became the basis for analytical procedures developed by McCready and Hassid (47), Kerr and Trubell (48) and others, for the determination of the composition of starch materials.

Rundle *et al.* (49) found that when half the amount of iodine required to form a complex with a starch sample is added to an amylose solution and the partial complex is isolated by precipitation with excess iodide, all of the iodine is in the precipitated amylose complex and none is left in solution. Thus, it appears that once iodine starts to fill a helix, it does so in preference to filling an empty one and that the tendency for iodine to leave a filled helix is much less than the tendency to leave one which is just starting to be filled. Furthermore, it is known from titration studies that iodine is taken up by longer helices at lower iodine activities than those required for shorter helices. It appeared from the titration of mixtures of potato and corn amyloses that the longer potato amylose titrated completely before the shorter corn amylose took up any iodine. Therefore, it seems evident that any condition which increases the number of iodine molecules arranged linearly within the amylose helix lowers the tendency of the iodine to escape from the helix, that is, increases the stability of the complex. Rundle, *et al.* have proposed that this stability is the result of induced dipoles. These authors point out that the glucose units comprising the helix consist of a number of dipoles. Those dipoles at right angles to the helix axis should oppose each other but unless the resultant dipole is exactly normal to the axis, a component will be directed parallel to the axis. Even though this value may be small it should increase with the length of the helix and it is probable that the value could assume large proportions for a very long helix. When iodine is introduced parallel to the axis it should acquire an induced dipole and succeeding iodine molecules should acquire parallel dipoles so that the induced dipole interactions should be attractions and the interactions of the induced dipoles should contribute to the strength of each. Thus, the increasing stability as helices are filled, and the greater stability of long helices filled with iodine compared to shorter ones are understandable.

Stein and Rundle (50) have elaborated on this conception and have critically examined the forces which may be expected to play a part in the formation of the starch-iodine complex. It was shown that additional stabilization may occur as a result of the formation of a resonating polyiodine chain at high dipolar interaction, having an I-I distance and heat of formation which compared favorably with experimental values.

Bear (51) has observed that a large number of substances, for example, alcohols, phenols, ketones and fatty acids, induce amylose molecules to assume a helical configuration and to precipitate with them in complex formation. To be sure, the diameter of the helix is not always the same in each case. The bushy shaped alcohols, such as secondary amyl, create a larger helix than do the normal alcohols. Bear pointed out that all of these complexing agents for amylose had one property in common which is readily apparent: possession of a permanent electric moment, or a tendency to assume an induced moment, as in the case of iodine.

It would seem probable that the reaction of amylose with iodine, and the resulting formation of a complex, is not unique but represents only one of a number of reactions for amylose which are quite similar in many respects. Because of the application in providing an effective fractionation technique, experiments concerning the treatment of starch with alcohols, such as butanol, have been adequately discussed in a previous chapter on composition and structure. The interaction of starch and fatty acids has received less attention.

Kerr and Trubell (48) observed that the trace amounts of fatty acids present in native corn starch seriously interfered with the formation of a complex between amylose molecules and iodine. They reported that by defatting corn starch the analytical value for amylose by spectrophotometric measurements rose to as high as 29%, whereas values of the order of 22% had been reported by other investigators using iodometric procedures on the native starch. Kerr and Trubell attributed this result to the insolubilizing effect of the fatty acids on the amylose. Kerr (52) had previously noted that fatty materials tended to insolubilize amylose from aqueous dispersions. Kerr (53) also reported that the subfraction of corn amylose which is isolated by insolubilization when the starch is treated with β-amylase, materially decreased as the starch was first defatted, and Schoch and Williams reported (54) that fatty acids could be used as fractionating agents for starch and extended the observed effect of fatty acids on amylose-iodine complex formation to potentiometric titration procedures. Mikus *et al.* (55) have studied the amylose-fatty acid complex in some detail. These investigators found that when solutions containing amylose were treated with either oleic, palmitic or lauric acid, the complex which formed gave a "V" pattern in x-ray studies and that the helices were packed together in very nearly the same manner as when iodine or alcohols, such as butanol, were used. Since the distances between helices were not altered measureably by the presence of the complexing agents it was concluded that the complexing agents must be placed in the holes in the crystalline structure. For the fatty acid complexes and the iodine complex, the amount of complexing agent bound by helical amylose could be explained satisfactorily in terms of the capacity of the helices. Thus, by an inspection of fatty acid complexes it was found that there were 17.6 glucose residues for each lauric acid molecule, 22.6 per palmitic and 25.0 per oleic acid molecule. Assuming a helical configuration for amylose, having a length of 8 A. per helix turn of six glucose residues, these ratios are proportional to the length of fully extended fatty acid molecules, including van der Waals distances, which are, 19 A. for lauric, 24 A. for palmitic, and 27 A. for oleic acid. Since the lily bulb amylose preparation which was used assayed 90% purity against the crystalline amylose of Kerr and Severson as a standard, then correcting for 10% amylopectin impurity which binds no fatty acid, the ratio of length of helix to length of fatty acid molecules bound would be unity in each case. Furthermore, it was found that on heating a dry, amylose-fatty acid complex in the presence of iodine vapor the fatty acid was displaced and iodine was taken up. From this it was concluded that the fatty acid occupied the same position in the structure of the complex as did the iodine, that is, along the long axis of the helix. The force between amylose and fatty acid was explained in terms of dipolar interactions.

Although the explanation which has been discussed above to account for complex formation between amylose and various complexing agents rationalizes the observed facts in a more adequate manner than other theories which have been proposed from time to time, one experimental approach which has been used to develop the helical amylose complex theory has, of necessity, been limited and this limitation, among others, has given rise to alternate explanations. The alternate theory most commonly advanced in the past has been that complex formation is an adsorption phenomenon between groups, possibly hydrophylic in nature, along the amylose chain and the complexing agent and that the stability of the complex is due to secondary forces (32, 33, 56-61).

In the treatment of dry amylose samples with complexing agents, experiments have been limited to the use of the "V", "A", and "B" forms. The latter two are the oriented states found in granular, retrograded, and frozen gel forms; they are insoluble in cold water and do not form complexes to any great extent even with iodine vapor. The "V" form is precipitated from solution by the addition of alcohols and is soluble in water. It is because this special form, the helical configuration does enter into complex formation to a very marked degree, whereas the other two do not, that the action is thought not to be simple adsorption. However, it seems reasonable to suppose that in the "A" and "B" configurations, the linearly oriented molecules are associated through their hydrophylic groups, which association no doubt accounts for their insolubility. That is, in these forms the groups which adsorption proponents suggest are active in complex formation are blocked because of associations already existing between these groups whereas in the water soluble "V" configuration this can hardly be the case, and it would be expected that these molecules would be better able to form adsorption complexes. Recently, Kerr and Katzbeck (62) have overcome the experimental limitation discussed by preparing an amylose sample in a completely water soluble form which also proved to be amorphous by x-ray studies. This was accomplished by rapidly drying the damp, Pentasol precipitate from starch between heated rollers. It was found that this amorphous, non-associated form of corn amylose was as inactive in the solid state with complexing agents as was corn amylose in retrograded and frozen gel forms, in contrast to the "V" forms of corn, potato and tapioca amyloses, which were highly reactive. Their results, when the four water soluble amylose samples, at a moisture content of 5.8%, were exposed to iodine vapor at room temperatures and pressures are shown in Fig. 99. It will be seen that the three amylose samples in the "V" form took up about 20% by weight of iodine vapor during the first 12 days, following which there was very little change. In contrast, the amorphous, water soluble form took up only about 4% of iodine and remained very nearly colorless. The behaviour of the retrograded form was practically the same as the amorphous; amylose in the frozen gel form took up slightly more.

In a similarly conducted experiment, corn and tapioca amyloses in the "V" form and retrograded and amorphous corn amylose, all at a moisture content of 6.1%, were exposed to butanol vapors at room temperatures and pressures. The two amyloses in the "V" form took up about 19% by weight

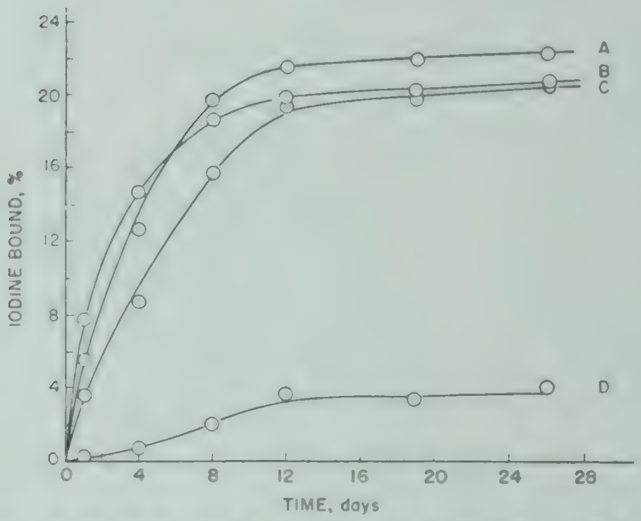


FIG. 99. Percent iodine taken up by amylose. *A*, *B* and *C* are V-forms from tapioca, corn and potato; *D* is amorphous.

of butanol vapors in about 30 days, whereas the retrograded and amorphous forms absorbed less than 0.5%. Additional 1-g. samples of the four, last mentioned amyloses were refluxed with 25 ml. of methanol containing 10 g. of oleic acid. Thereafter, they were thoroughly extracted with carbon tetrachloride and hydrolyzed with acid to determine the amount of fatty acid combined with the amyloses. The two amyloses in the "V" form took up 2% of oleic acid whereas the amorphous form absorbed 0.1% and the retrograded, 0.3%.

It is apparent, therefore, that the presence of free hydroxyl groups on the amylose chains is not the prime requisite for complex formation but rather, a special configuration of the amylose molecules is the necessary condition. Present data, as mentioned earlier, indicates that the "V" form is a helical configuration.

In passing, it is of interest to note that intensely dried amyloses samples of 2% or less moisture content either take up very much less of the three complexing agents discussed above, or combine at a very greatly retarded rate.

2. Reaction of Starch with Formaldehyde and other Cross-Linking Reagents. The action of aldehydes on starch is probably quite complex and is not thoroughly understood at present. It is quite likely that the reaction proceeds by several mechanisms, or stages, depending on the concentration of the particular aldehyde used, the extent to which the reaction is permitted to take place, and other conditions, such as the acidity and the alkalinity of the medium and the addition of other catalysts. Aldehydes appear to react differently with starch in the presence of strong alkali and in the presence of ammonia and urea. This discussion is not primarily concerned with these latter reactions but it will treat mainly of the less complex system, aldehyde and starch.

If a starch paste is treated with a 40% formalin solution, it will be observed that the ability of the starch to stain blue with iodine will gradually disappear, the extent depending on the amount of formaldehyde added. The color with iodine changes from a blue, through purple, to red, and finally to yellow, which indicates the final disappearance of the coloration. This effect is so strikingly similar to the action of acid and enzymes in degrading starch through the various dextrin stages, that early workers, such as Woker (63) and Maggi (64), were led to believe that the action of formaldehyde is a hydrolytic dextrinization. The theory was found to be weak, for Maggi noted that the viscosity increased during the reaction and that the specific rotation of the solution did not decrease proportionately to the color change, as is noted in reactions in which hydrolytic agents are used. Maggi suggested that the aldehyde can degrade starch only to a dextrin stage and that these dextrins exhibit a decidedly different volume contraction from that of the diastatically made dextrins.

Syniewski (65) proposed that the hydrolytic action of the formaldehyde might be a carbinol hydrolysis, which accounts for the fact that the end-products do not reduce Fehling's solution.

Jacoby (66) showed, however, that the conclusions drawn from changes in iodine coloration were not valid, since after starch has been allowed to react with aldehyde until it has lost its ability to produce a color with iodine, the coloration may be progressively restored by the addition of ammonium acetate, whereby the aldehyde is removed from the medium (and possibly also the starch) to form hexamethylenetetramine. That little degradation in the starch has occurred during the formaldehyde reaction is confirmed by the work of von Kaufman (67, 68). If alcohol is added, the starch is precipitated in the same state as it is obtained by adding alcohol to untreated starch pastes. With mild conditions for the reaction of formaldehyde, such as treating whole starch with 40% formalin at room temperature, and provided the reaction has not been too extensive, the iodine coloration given by starch may be restored simply by the addition of large quantities of water. In other cases, the addition of an acid will facilitate the ability of the product to form a blue color with iodine (65).

Woker attempted to defend his theory by suggesting that a resynthesis of the starch degradation products occurs under certain conditions, which synthesis may have occurred during the experiments of other workers (69, 70). However, Samec and Mayer (71) could find no evidence for a degrading or resynthesizing action. The reaction was studied under various conditions with starch and its amylose and amylopectin fractions. In all cases, a highly hydrated compound (or complex) is formed, which finally gives no color with iodine and which is of the same order of molecular magnitude as before treatment. For starch so treated the dialyzable fraction remains about the same, but the viscosity is higher and the optical rotation is only slightly lower than that for the original starch. The conclusion was drawn that a loose addition product is formed. Samec also made the significant observation that the increase in viscosity which results from the addition of formaldehyde reaches its peak, in all cases, at the point where the iodine coloration disappears.

Samec and other workers have likened the physical action of formaldehyde on starch to that of alkali. Samec has noted that, if potato starch is treated with formaldehyde freshly distilled over $CaCO_3$, the granules swell and form an exceptionally thick gel. The volume of the gel constantly increases until it finally distributes itself completely throughout the liquid to produce a very viscous solution.

If aldehydes of less activity, such as acetaldehyde, are added to starch solution, on aging there is no loss in the ability of the sol to form a blue color with iodine, which loss otherwise normally occurs. But if the acetaldehyde is removed, the normal decrease in such ability begins at once. Normally, starch solutions lose their ability to color blue with iodine owing to a retrogradation effect. The linear chain molecules become intermolecularly cross-bonded, possibly by hydrogen bonding, and it thus becomes more difficult for these chains to assume a helical shape around the iodine molecules, as described in the previous section. That the action of aldehyde is to prevent retrogradation was shown by Samec (72). Whereas 1% solutions of amylose change within a short time to milky liquids and at higher concentrations quickly coagulate, 1% amylose solutions remain clear and stable in the presence of added formaldehyde. With a sufficient concentration of the latter, however, the amylose loses its ability to color blue with iodine.

The more advanced stages of the starch-formaldehyde reaction have been studied by Classen (73). Starch treated with formaldehyde at higher temperatures produces finally a compound from which the excess aldehyde may be removed by boiling in water or by washing the product with a solution of sodium bisulfite. The starch product is insoluble in the hot water and hence may be isolated. It shows a constant composition of 1 mole of formaldehyde to 1 mole of starch and remains stable even when heated to 180°C.

From the available facts, the mechanism of the action of aldehyde on starch might be outlined as follows. In the initial stages of the action the aldehyde may enter into the original orientation within the granule to form a complex with hydroxyl groups where hydrogen bonding is weakest or where it does not exist. As the reaction proceeds, the molecular structure so opened up tends to swell, since, after a sufficient number of hydroxyl groups have become associated with the aldehyde, the network of the molecules becomes enlarged and in addition water molecules can now enter into the interiors of these orientations. If the starch has been pregelatinized, as by heating in water, and the network is opened up through association with water molecules, then it would seem that some of these water molecules either are replaced by the formaldehyde molecules or that the hydroxyl groups of the carbohydrate associate first with the aldehyde and then perhaps with the water present. Evidently only by a "blocking" effect at these hydroxyl groups could the direct association of linear chains (or linear sections) be prevented as the paste ages. As the linear chains become loaded with aldehyde groups, their ability to form helical coils around iodine molecules is decreased, and the intensity of the blue coloration with iodine is accordingly decreased also. The three-dimensional network of the branched chains becomes opened and they decrease in their ability to absorb and hold iodine within their interiors. The type of association discussed is evidently weak and easily reversible, but, as the reaction proceeds, it seems probable that a more stable bond is formed. Possibly cross-bonds are formed from a hydroxyl group on one chain through a methylene group to a hydroxyl group on another chain. This would increase the apparent viscosity and in some cases the gelling properties of the system and decidedly lessen the tendency of linear chains to orient with iodine in much the same manner as direct cross-bonding of hydroxyl groups accomplishes the same results in the early stages of retrogradation. Finally, the number of stable methylene cross-links per carbohydrate molecule becomes such that the starch loses its ability to swell in hot water, becomes increasingly resistant to acid and alkali and is stable when heated to comparatively high temperatures, just as is highly retrograded starch.

From a study of the action of aldehydes on the building units of proteins, a theory of cross linking through methylene groups as proposed by Meyer (74), between such reactive groups as amines, imines and amides, has been developed by Fraenkel-Conrat and co-workers (75-80) and others, to explain the action on the polymer. The links are believed to form in neutral or acid media and to be relatively acid resistant. French and Edsall (81) have discussed the tanning action of formaldehyde on proteins such as collagen and casein on the basis of methylene cross-links, which explains the lesser tendency of the proteins to swell in water and their increased resistance to acid and alkali.

Fundamental studies on carbohydrate building units, by Sutra (82) has not, however, given support to the cross-link theory of action by aldehydes. In the case of glucose, in acid media, Sutra reported that paraldehyde reacted to form a mixture of monoethylidene derivatives and a small amount of 4,6-ethylideneglucose was separated. Maltose was found to give a non-reducing diethylidene maltose, insoluble in cold water.

However, starch chemists appear generally to have accepted the proposal of Maxwell (83) that the unusual transition which starch undergoes in paste properties when treated with bifunctional reagents such as epichlorohydrin, or dibasic acids, is best understood on the basis of the formation of cross-links between the polymer molecules. With a very limited treatment, the paste viscosity of the starch increases and the paste is remarkably stable to heat. As the number of cross-links become appreciable, however, the paste viscosity becomes less, owing to a lesser tendency of the granules to swell and eventually they lose their ability to gelatinize by heating in water and become more resistant to acid and alkali. A comparable transition may be noted by treating starch with formaldehyde. The results parallel the changes induced in the properties of high polymers in general by the creation of cross-links, as discussed by Flory (84) and by Mark (85).

Considerable data now exists which favor the view that reagents which are bifunctional, or potentially bifunctional under the proper conditions, such as sodium tetraborate, but which have functional groups of low activity in respect to starch, increase the paste viscosity and otherwise modify its colloidal properties by the establishment of weak cross-links. Isbell and coworkers (86) have recently found that either sodium or potassium tetraborate acts on glucose to form a diglucose borate. This compound is relatively labile, dissociating to the monoglucose borate with changes in concentration of the reactants.

In the case of carbohydrate polymers, the tetraborate cross-links are presumed to form and dissociate continuously and this dynamic state is thought to explain the unique mechanical properties of their gels, or sols, such as ropiness (87).

Quite possibly other polyhydroxy acid groups act by a similar cross-linking mechanism to a greater or lesser extent. Samec (88) has reported very substantial increases in the viscosity of potato amylose by the introduction of phosphoric acid groups and it may very well be that some of the unusual properties of the naturally occuring potato amylopectin are explainable on the basis of phosphate, or diphospho cross-links which are relatively labile. Griffin, *et al.* (89) have disclosed that starch forms a water-insoluble complex when heated with potassium pyroantimonate under acidic conditions. Caesar (90) has insolubilized starch with chromate.

Thus, the action on starch of formaldehyde, and other lower aldehydes, is no longer thought to be unique, but rather to be representative of a general class of reactions, the cross-linking of carbohydrate polymer molecules.

Some of the applications of the aldehyde reaction have been noted in the chapter concerning modified starches, particularly the utilization of the products of the early stages of the reaction. Starches of rather high viscosity, which find some application as sizes in paper manufacture* and in certain types of adhesives, are produced. The extended action of aldehydes on the non-cereal starches, such as potato and tapioca, to induce them to form firm, transparent gels, that is, to inhibit their dissolution in water, has been reported by Kerr and Schink (91). Möller (92) has patented the production of a swelling starch by treatment with formaldehyde. Bauer and coworkers (93) have treated dry dextrins with paraformaldehyde at high temperatures to reduce the swelling, dispersibility, or solubility of the product in water.

Additional application of the reaction in its more extended phases are to be noted in the patent literature. Classen (94, 95) has patented the use of his insolubilized starch, mentioned above, as a surgical dressing. Pierson (96) has patented a starch product made by heating the granules with 3 to 10% of formaldehyde and acid at pH 2.5 to 4.0, for from 2 to 24 hours at temperatures between 150 and 220°F. The starch does not gelatinize in boiling water or aqueous alkali. Blumer (97) and others (98-100) have described the manufacture of both water-soluble and water-insoluble adhesives and plastics. Weiss (101) has claimed a launderable textile size.

Some of the more important applications of the formaldehyde reaction are those in which other condensing agents are also present with the starch. Phenols, ammonia, urea, melamine, and other bases are frequently employed in production of water-insoluble, or water-resistant, starch sizes and adhesives. Very likely in these cases there is a competitive reaction between the starch and the other reagent for the aldehyde. Little is known of the extent to which starch participates in many of these applications. Native, acid-modified thin boiling and enzyme-converted starches and dextrins have been used in these reactions or modifications.

Loetscher (102) produced a water-soluble resin by adding 5 parts of 5% NaOH to 100 parts of water containing 100 parts of carbohydrate, followed by the addition of 100 parts of phenol and 100 parts of 40% formaldehyde. The mass is heated to 200-225°F. and held at this level until it becomes clear and viscous, but the heating is stopped just before the resin becomes water-insoluble. Berquist (98) treated thin boiling starch with formaldehyde and an ammonium compound, which was then further hydrolyzed to produce a clear, viscous adhesive, as follows: A mixture of 190 g. of corn starch, 225 cc. of water, 0.3 g. of NH_4Cl, 0.65 g. of formaldehyde (40%), and 0.5 cc. of HCl (23° Bé.) is stirred for 30 minutes and filtered. The product is dried, first at a low temperature and then at increasing temperatures until 170°F. is reached. The starch product is held at this temperature until the desired viscosity is attained. Leuck (99) dextrinized starch in the presence of ammonia, and then treated the product with formaldehyde and acid to produce a water-resistant adhesive.

---

*See Chapter III, Manufacture of Modified Corn Starches, particularly starches of high viscosity.

The recent trend to conserve wood in the manufacture of containers for food products, general merchandise, and munitions by use of water-resistant paper board, fabricated from laminated paper, is facilitated by the use of water-resistant sizes and adhesives made from starch products, urea, and formaldehyde (103). Thick starches and dextrins or combinations of the two are heated in the presence of a urea and formaldehyde, or a product which liberates formaldehyde, until the starch product is dispersed in the water present. Either an ammonium salt or a weak acid, or a combination of both, may be added to catalyze the condensation which follows after the size or adhesive is applied. With applied heat, or after an aging at lower temperatures, the product develops insolubility to water. Several formulae are currently used which produce surprisingly water-resistant, laminated paper board. Taft (103) has discussed some of the properties of urea-formaldehyde resins and their application as adhesives in paper manufacture. The application or use of starch products in this type of formulation will be discussed further in Chapter XXII on adhesives.

Urea-formaldehyde condensation products are also used with starch to produce paper sizes with increased strength and water resistance. The application may be made at the beaters or in tub application, or the resin may be added to starch-clay coating mixtures to impart wet rub resistance. Melamine, or other bases are also used in place of urea. In order to produce a tougher starch gel which is precipitable by alum, Fowler and Pattilloch (104) recommend the addition of sodium metasilicate to the urea-formaldehyde reaction mixture with starch.

3. Hydrogenation of Starch. Of the common reactions applied to organic substances, hydrogenation has been the least studied in the case of starch. The reluctance of investigators to study the hydrogenation of starch products arises no doubt from the many possible coreactions which may follow depending upon conditions. Thus, when starch is treated with hydrogen at high pressures and temperatures, it would appear that depending on the pressure, temperature, time, catalyst, solvent and the acidity, the following reactions are possible:

(a) Hydrogenation of carbonyl groups, such as aldehydes, to hydroxyls.
(b) Hydrogenolysis of glycoside linkages between sugar groups with the formation of hexitols.
(c) Or, hydrolysis of glycoside linkages followed by reduction of aldehydes by hydrogenation.
(d) Hydrogenolysis of carbon to carbon valences, such as, for example, the splitting of hexitols, as formed in (b) or (c) to lower glycols such as glycerol.
(e) Hydrogenation of glycols with a reduction of one or more alcohol groups to alkyl groups such as, for example, the reduction of glycerol to propanediol-1,2, or of ethylene glycol to ethyl alcohol.

Ipatieff (105) hydrogenated glucose and obtained a quantitative yield of sorbitol when 100 atmospheres of hydrogen were used at 130°C. in the presence of nickel. Other workers (106-108) subsequently obtained sorbitol from glucose at comparatively low temperatures (under 200°C.) with various catalysts. Thus, it may be presumed that in the presence of water or moisture, and slightly acidic conditions, starch would be hydrolyzed to glucose and the first phase of the reaction, reduction to hexitols, would proceed with moderate ease. Rigamonti and Beati (109) reported that corn starch and dextrin are quantitatively converted to sorbitol at 170°C. with 200 atmospheres pressure of hydrogen and that nickel and kieselguhr are the best catalysts. The reaction with soluble starch was found to proceed more rapidly in the presence of 1% phosphoric acid.

A continuous process for the production of sorbitol, in a specially designed reactor, has been outlined by Porter (110). Flexser (111) has found that sugars are very much more readily reduced to polyhydric alcohols by adding 10%, by weight, of Mg turnings to the Raney Ni catalyst. For example, when 15 g. of Mg. turnings was added to 150 g. of Raney Ni, the mixture converted 1.5 kg. of glucose in 3.1 liters of water quantitatively to sorbitol in 15 minutes at 85-95°C. and 20 atmospheres pressure of hydrogen. The catalyst with the Mg turnings may be reused indefinitely, by adding 5 g. of Mg to every tenth batch.

From a study of thermodynamic data, Beati, *et al.* (112) have found that the hydrogenolysis of sugars involving the rupture of C-C valences proceeds with greatest ease between carbons 3 and 4. Natta, *et al.* (113) found that this was particularly true when copper catalysts were used but that nickel promoted the less thermodynamically favorable reaction in which splitting is between carbons 2 and 3. Furthermore, it was found that the very active nickel catalysts were less suitable than copper-nickel catalysts with polysaccharides such as starch or dextrin, owing to the sensitivity of the nickel catalyst to the acidity of the partially hydrogenated solution oi starch or dextrin. Schmidt (114) found that regardless of whether 4, 5, or 6 carbon polyhydric alcohols were hydrogenated, propylene glycol was the principal product, added evidence of the tendency of these polyhydric alcohols to split between carbons 3 and 4.

The results of Natta and coworkers (113) are in agreement with thermodynamic considerations which show that hydrogenation reactions in which water is formed are most favored and of these, the reduction of primary alcohols are favored over the reduction of secondary alcohols. Thus, there is a tendency following hydrogenolysis of hexitols to glycerol for the latter to be reduced to propanediol,-1,2 and then to isopropyl alcohol, rather than to *n*-propyl alcohol. Connor and Adkins (115) found that glycerol was converted almost quantitatively into propylene glycol by using copper chromite catalyst at 200°C. for 4 hours. A patent to Du Pont (116) for the production of propylene glycol claims the use of a nickel catalyst together with an oxide such as, Cr_2O_3, Al_2O_3 or ThO_2, which favors the removal of water molecules.

A consideration of free energy changes for the types of reactions mentioned above would indicate that the hydrogenation of hexitols to methyl pentitol should occur in preference to the hydrogenolysis of hexitol to glycerine and the reduction of glycerine to propylene glycol. Furthermore, Natta has pointed out that the splitting of methyl groups from 1-methyl polyhydric alcohols is thermodynamically favored by a wide margin over splitting of C-C valences at other positions. However, Natta has pointed out that the nature of the catalyst employed has considerable influence on determining the reaction which will predominate and that copper catalysts are preferred for avoidance of methane formation from hexitols through the intermediate, methyl pentitol.

Adkins (117) and coworkers have shown that glucose, sorbitol and manitol yield about the same products in substantially the same amounts when hydrogenated with copper chromite at 250°C. for a period of 2 hours. At the end of this time the yields were about 50% propylene glycol, 15% hexitols, 7% ethanol and 3% methanol. However, at lower temperatures and using copper chromite in an alcoholic media, Natta and coworkers (113) found that neither monohydric alcohols, ethylene glycol, nor methyl glycerol were produced from rice starch, dextrin or glucose. The products found were principally propylene glycol, sorbitol, and glycerol. Under certain condition, yields of propylene glycol as high 70% were obtained and this was increased to 80% by subjecting the high boiling point residues to a second hydrogenation. In aqueous media the yield was found to be about 30% propylene glycol and 20% glycerol, particularly with nickel catalysts, and these yields were increased to 45% and 30%, respectively, by rehydrogenating the hexitol residues. Raney nickel was found to be unsuitable at higher temperatures.

Therefore, the proportion of the various products obtained will depend to a large extent on the conditions employed which favor the reactions desired and repress those which promote unwanted secondary or side reactions. For the production of glycerol or glycols in high yield, for example, both Du Pont (118) and Natta (113) propose a two, or multistage hydrogenation. The former recommends a preliminary hydrogenation at low temperature to obtain the hexitol, followed by a high temperature hydrogenation with the addition of $CaCO_3$ which promotes the formation of glycol. Natta proposes a preliminary hydrogenation at 130-160°C., followed by a secondary hydrogenation at 220-250°C., preferably with copper chromite in alcoholic solution. Using the latter, yields of glycerol may reach 30-40%, and in a three stage process, 50%.

Utilization of the hydrogenation reaction need not necessarily be confined to its use as a primary modification of starch. In addition to hydrogenation of products of hydrolysis such as sirups and sugars, other preliminary modifications suggest themselves, for example, oxidation and dextrinization. When controlled oxidation is first used, subsequent hydrogenation, or hydrogenolysis, would lead to the production of unusual glycols or hydroxy acids, depending on the type and extent of the preoxidation. The use of hydrogenation to convert undesirable carbonyl groups to non-reducing groups in pyrodextrins has been patented (119).

BIBLIOGRAPHY

1. de Claubry, G., *Gilbert's Ann. Phys.,* **48**, 297 (1814).
2. Leroy, *Phil. Mag.,* **4**, 313 (1834).
3. Stocks, H. B., *Chem. News,* **57**, 183 (1888).
4. Mylius, F., *Z. physiol. Chem.,* **11**, 306 (1887).
5. Roberts, C. F., *Am. J. Sci.,* **47**, 422 (1894).
6. Hale, F. E., *J. Chem. Soc.,* **84**, 151 (1903).
7. Harrison, W., *Kolloid-Z.,* **9**, 5 (1911).
8. Pickering, S., *Chem. News,* **42**, 311 (1880).
9. Vogel, A., *Neues Repert, Pharm.,* **25**, 565 (1876).
10. Radley, J. A., Starch and Its Derivatives, New York, 81 (1940).
11. Phol, J. J., *J. prakt. Chem.,* **83**, 35 (1861).
12. Payen, A., *Compt. rend.,* **61**, 466 (1865).
13. Payen, A., *Compt. rend.,* **61**, 1021 (1865).
14. Pinnow, J., *Z. anal. Chem.,* **41**, 485 (1902).
15. Harrison, W., *Kolloid-Z.,* **9**, 5 (1911).
16. Kolthoff, J. M., *Chem. Centrl.,* **2**, 717 (1919).
17. Schaer, E., *Pharm. Centralhalle,* **37**, 540 (1896).

18. van Deventer, C. M., *Chem. Centrl.*, 424 (1888).
19. Schoenbein, C. F., *J. prakt. Chem.*, **84**, 402 (1861).
20. Rouvier, G., *Compt. rend.*, **124**, 565 (1897).
21. Toth, J., *Chem.-Ztg.*, **15**, 1523, 1583 (1891).
22. Padoa, M., and Savarè, B., *Atti Accad. Lincei* **14**, 467 (1905).
23. Andrews, L. W., and Goettsch, H. M., *J. Am. Chem. Soc.*, **24**, 865 (1902).
24. von Euler, H., and Myrbäck, H., *Ann.*, **428**, 1 (1922).
25. Lottermoser, *A., Z. angew. Chem.*, **34**, 427 (1921).
26. Lottermoser, A., *Kolloid-Z.*, **33**, 271 (1923).
27. Rodewald, H., and Kattein, A., *Sitzber. preuss. Akad. Wiss.*, 628 (1899).
28. Friedenthal, H., *Centr. Physiol.*, **13**, 54 (1899).
29. Katayama, M., *Z. anorg. Chem.*, **56**, 209 (1908).
30. Dhar, N. R., *J. Phys. Chem.*, **28**, 125 (1924).
31. Berczeller, L., *Biochem. Z.*, 133, 502 (1922).
32. Hanes, C. S., and Cattle, M., *Proc. Roy. Soc. London*, B125, 387, 414 (1938).
33. Hanes, C. S., *New Phytologist*, **36**, 189 (1937).
34. Freudenberg, K., Schaaf, E., Dumpert, G., and Ploetz, T., *Naturwissenschaften*, **27**, 850 (1939).
35. Maquenne, L., and Roux, E., *Compt. rend.*, **140**, 1303 (1905).
36. Kerr, R. W., and Severson, G. M., *J. Am. Chem. Soc.*, **65**, 193 (1943).
37. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 558 (1943).
38. Rundle, R. E., and French, D., *J. Am. Chem. Soc.*, **65**, 1707 (1943).
39. Rundle, R. E., and Edwards, F. C., *J. Am. Chem. Soc.*, **65**, 2200 (1943).
40. Rundle, R. E., and Baldwin, R. R., *J. Am. Chem. Soc.*, **65**, 554 (1943).
41. Murray, H. D., *J. Chem. Soc.*, **127**, 1288 (1925).
42. Bates, F. L., French, D., and Rundle, R. E., *J. Am. Chem. Soc.*, **65**, 142 (1943).
43. Foster, J. F., Dissertation, Iowa State College, Ames, Iowa (1943).
44. Dube, H. A., Dissertation, Iowa State College, Ames, Iowa (1947).
45. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.*, **66**, 111 (1944).
46. Simerl, L. E., and Browning, B. L., *Ind. Eng. Chem., Anal. Ed.*, **11**, 125 (1939).
47. McCready, R. M., and Hassid, W. Z., *J. Am. Chem. Soc.*, **65**, 1154 (1943).
48. Kerr, R. W, and Trubell, O. R., *Paper Trade J.*, **117**, 25 (1943).
49. Rundle, R. E., Foster, J. F., and Baldwin, R. R., *J. Am. Chem. Soc.*, **66**, 2116 (1944).
50. Stein, R. S., and Rundle, R. E., *J. Chem. Phys.*, **16**, 195 (1947).
51. Bear, R. S., *J. Am. Chem. Soc.*, **66**, 2122 (1944).
52. Kerr, R. W., *Paper Trade J.*, (TAPPI), **115**, 30 (1942).
53. Kerr, R. W., *Cereal Chem.*, **20**, 299 (1943).
54. Schoch, T. J., and Williams, C. B., *J. Am. Chem. Soc.*, **66**. 1232 (1944).
55. Mikus, F. F., Hixon, R. M., and Rundle, R. E., *J. Am. Chem. Soc*, **68**, 1115 (1946).
56. Meyer, K. H., and Bernfeld, P., *Helv. Chim. Acta*, **24**, 389 (1941).
57. Lehrman, L., *J. Am. Chem. Soc.*, **64**, 2144 (1942).
58. Whistler, R. L., and Hilbert, G. E., *J. Am. Chem. Soc.*, **66**. 1721 (1944)
59. Schoch, T. J., *Advances in Carbohydrate Chem.*, **1**, 247 (1945).
60. Schoch, T. J., *Baker's Digest*, **21**, No. 1, 1 (1947)
61. Schoch, T. J., in Starch and Its Derivatives, by J. A. Radley, 3rd ed., in press
62. Kerr, R. W., and Katzbeck, W. J., Complexes of Amylose, Manuscript in preparation.
63. Woker, G., *Ber.*, **49**, 2311 (1916).
64. Maggi, H., *Fermentforschung*, **2**, 302 (1919).

65. Syniewski, W., *Ann.,* **324**, 201 (1902).
66. Jacoby, M., *Ber.,* **52**, 558 (1919).
67. von Kaufman, W., *Biochem. Z.,* **78**, 371 (1917).
68. von Kaufman, W., *Ber.,* **50**, 198 (1917).
69. Woker, G., and Maggi, H., *Ber.,* **52**, 1594 (1919).
70. Woker, G., *Biochem. Z.,* **99**, 307 (1919).
71. Samec, M., and Mayer, A., *Kolloid-Beihefte,* **13**, 165 (1921).
72. Samec, M., Kolloidchemie der Stärke, Dresden, 434 (1927).
73. Classen, A., *Therap. Monatsh.,* **33** (1897).
74. Meyer, K. H., *Biochem. Z.,* **208**, 23 (1929).
75. Fraenkel-Conrat, H. L., Cooper, M., and Olcott, H. S., *J. Am. Chem. Soc.,* **67**, 950 (1945).
76. Fraenkel-Conrat, H. L., and Olcott, H. S., *J. Am. Chem. Soc.,* **68**, 34 (1946).
77. Fraenkel-Conrat, H. L., and Olcott, H. S., *Federation Proc.,* **6**, 253 (1947).
78. Fraenkel-Conrat, H. L., Brandon, B. A., and Olcott, H. S., *J. Biol. Chem.,* **168**, 99 (1947).
79. Fraenkel-Conrat, H. L., and Olcott, H. S., *J. Am. Chem. Soc.,* **70**, 2673 (1948).
80. Fraenkel-Conrat, H. L., and Olcott, H. S., *J. Biol. Chem.,* **174**, 827 (1948).
81. French, D., and Edsall, J. T., *Advances in Protein Chem.,* **2**, 317 (1945).
82. Sutra, R., *Bull. soc. chim.,* **9**, 794 (1942).
83. Maxwell, R. W., U. S. Patent 2,148,951 (1939).
84. Flory, P. J., *J. Am. Chem. Soc.,* **58**, 1877 (1936); **61**, 3334 (1939); **62**, 1057, 2255 and 2261 (1940).
85. Mark, H., *Ind. Eng. Chem., Ind. Ed.,* **34**, 1343 (1942).
86. Isbell, H. S., Brewster, J. F., Holt, N. B., and Frush, H. L., *J. Research Nat'l Bur. Standards,* **40**, 129 (1948).
87. Duel, H., Neukom, H., and Weber, F., *Nature,* **161**, 96 (1948).
88. Samec, M., Kolloidchemie der Stärke, Dresden, p. 24 (1927).
89. Griffin, I., Truax, D. E., and Nuttall, N. H., U. S. Patent 2,413,320 (1946).
90. Caesar, G. V., U. S. Patent 2,111,204 (1938).
91. Kerr, R. W., and Schink, N. F., U. S. Patent 2,438,855 (1948).
92. Möller, F. A., German Patent 737,568 (1943).
93. Bauer, H. F., Bauer J. V., and Hawley, D. M., U. S. Patent 2,396,937 (1946).
94. Classen, A., German Patent 92,259 (1896); U. S. Patent 602,697 (1898).
95. Classen, A., German Patent 94,282 (1896).
96. Pierson, G. G., U. S. Patent 2,417,611 (1947).
97. Blumer, E., German Patent 179,590 (1904).
98. Berquist, C., U. S. Patent 1,287,841 (1918).
99. Leuck, G. J., U. S. Patents 2,222,872-3 (1940).
100. Herstein, B., U. S. Patent 982,673 (1911).
101. Weiss, E., U. S. Patent 2,411,818 (1946).
102. Loetscher, E. C., U. S. Patent 1,959,433 (1934).
103. Taft, P. B., *Paper Trade J.* (TAPPI) **115**, 206 (1942).
104. Fowler, G. B., and Pattilloch, D. K., U. S. Patent 2,385,438 (1945).
105. Ipatieff, W., *Ber.,* **45**, 3225 (1912).
106. Cake, W. E., *J. Am. Chem. Soc.,* **44**, 859 (1922).
107. Covert, L. W., Connor, R., and Adkins, H., *J. Am. Chem. Soc.,* **54**, 1651 (1932).
108. Yoshikawa, K., *Sci. Papers Inst. Phys. Chem. Research* (Tokyo) **25**, 235 (1934).
109. Rigamonti, R., and Beati, E. B., *Rend. inst. lombardi, sci.,* **73**, 633 (1939-40).

110. Porter, R. W., *Chem. Eng.,* **54**, 114 (1947).
111. Flexser, L. A., U. S. Patent 2,421,416 (1947).
112. Beati, E. B., and Natta, G., *Ricera sci.,* **14**, 15 (1943).
113. Natta, G., Rigamonti, R., and Beati, E., *Ber.,* **76B**, 641 (1943).
114. Schmidt, O., *Z. physik. Chem.,* (**A**)**159**, 337 (1932).
115. Connor, R., and Adkins, H., *J. Am. Chem. Soc.,* **54**, 4679 (1932).
116. E. I. du Pont de Nemours Co., U. S. Patent 1,963,997 (1929).
117. Adkins, H., Reactions of Hydrogen, Madison, Wis. (1937).
118. E. I. du Pont de Nemours Co., U. S. Patent 2,004,135 (1932).
119. I. G. Farbenindustrie Aktiengesellschaft, French Patent 852,033 (1939).

SECTION V. USES AND ANALYSIS

Section Five. Uses and Analysis

The uses for starch are as numerous and varied as are the sources from which it is derived. The task of cataloguing and detailing the properties of the innumerable botanical types of starch is no more difficult than tracing the utilization, or application of the product, as it affects each of us throughout every hour of our daily life. Starch may be used in the manufacture of soaps and other cleansers. It is used in the manufacture of the clothes we wear and in the laundering which keeps them serviceable. Starch, in some modification, or as a derivative, is an important constituent in the morning breakfast. It has been used at the foundry in the production of the vehicle which transports us to our work and returns us to our homes. Starch has no doubt entered into many of the manufactured structural elements or into the building operations used to make the place in which we work and the home in which we live. It is used in the manufacture of paper, such as writing paper, and paper products, such as boxes, containers, and labels, without which modern commerce would be at a serious disadvantage. Starch may be used in the manufacture of the furniture and rugs which equip our offices and homes. It is used in the preparation of soups, sauces, meat products, sirups, puddings, cakes, and a host of other items which make up the daily menu. Starch, or a derivative, is used in making candy, ice cream, ices, and some of our more popular beverages, both alcoholic and non-alcoholic. The largest single application for the production of a product for human consumption is in the manufacture of beer. Starch is used in the manufacture of such textiles as towels, table cloths, and bed linen, which we have considered indispensable to modern life. Accordingly, it will be noted that products the manufacture of which involves the use of starch products, are useful in our daily routine from the time we arise in the morning until we retire at night.

Sometimes these uses are obvious whereas in others the important part starch plays in the industrial operations in which they were made is not readily appreciated nor very well known generally. For example, the oil which may heat our home, supply the power to operate our business and may have been the source from which the motor fuel was derived that carried us to work may very well have been impossible or uneconomical to produce without the use of a modified starch product. Starch has entered into the refining of ores, such as aluminum, and processes have been patented recently for the refining of ferrous, or more properly ferric ores, such as hematite, and into the production of rubber goods, pharmaceuticals and plastics.

A complete discussion of all the many applications for starch products must be left to a more comprehensive work on this particular phase of the subject. In the following chapters an outline is presented first of those uses which have been traditionally considered to be the most important. These happen to represent as classes, the applications in which the largest volume of starch has been used. They are, the manufacture of paper, textiles and adhesives and the fermentation and food industries. Following these discussions a chapter has been added in which many other uses are briefly treated. Although some of these applications are relatively new they already show promise of rivalling in time the longer known and presently larger outlets for starch products. A table is also provided in this chapter in which uses for starch or its modifications have been summarized.

In a concluding chapter, analytical methods which apply particularly to starch have been reviewed. These include the analysis of products or sources for starch content, the analysis of starch for non carbohydrate materials common to commercial varieties and methods for the examination of starch to determine the manner and extent to which it may have been physically or chemically modified.

CHAPTER XVIII

USE OF STARCH IN PAPER MANUFACTURE

1. **Introduction.** Judged by the volume of starch consumed, the paper industry is one of the most important, non-food outlets for starch and starch products. Starch is used in various sizing operations during the manufacture of paper sheets and paper boards, and also in fabricating these into various containers and cardboard articles. In the latter cases, the starch product is used more as an adhesive, as in joining two or more sheets to form a laminated or corrugated board, to manufacture various kinds of boxes, bags, envelopes, cartons, and other containers. Although these manufacturing operations are an integral part of the paper industry, nevertheless, the use of starch products in these instances will be dealt with under the general subject of adhesives. This division in subject matter is purely arbitrary, inasmuch as an important function of starch in sizing is to create a bond between units in the paper; that is, act in the nature of an adhesive. However, this present section will be devoted to discussing the function and use of starch products in the various operations employed to form and finish the sheet of paper.

In the manufacture of paper, sizing is accomplished in one or more of three phases of the manufacture. It is added to the pulp, at the beaters, before the sheet is formed; it is applied as a so called surface size, as for example, tub sizing; and, lastly, certain papers, such as high grade magazine papers, are sized with coatings in which clay or other pigment is usually incorporated to produce a sheet which will have better characteristics for printing. Each type of sizing will be dealt with separately.

Paper is made from a pulp consisting essentially of cellulose fibers obtained from various sources. For the better grades of writing paper, in which durability is desired in respect to fold and tear, a part of the pulp is of cotton origin. Old rags are bleached and disintegrated to furnish a proportion of long fiber. Intermediate grades of white paper are made essentially by chipping certain varieties of wood, digesting the chips in caustic soda, sulfite, or other hydrolytic agents to remove lignin and other undesirable constituents, and, finally, bleaching. In the manufacture of wrapping and bag paper, bleaching is usually omitted. The lowest grades of paper contain substantial quantities of ground wood which, as might be expected, contains mostly short fiber. Certain paper products, such as paper board of the corrugated type or wall board used in building insulation, may contain substantial amounts of reclaimed paper, straw fiber, sugar cane fiber, or fiber from other sources.

2. Beater Sizing. After the crude pulp has been digested, washed, and bleached, if a light colored sheet is to be made, it may be dried and stored for future use or it may be passed on immediately to the beaters. Here the pulp is disintegrated further and dispersed into a relatively large amount of water by mechanical action. One object is to separate individual fibers so as to form a uniform sheet (or web, as it is called) when the pulp is collected on a screen filter, referred to as the "wire." Another object is to "hydrate" the fiber to the desired degree. Beating operations vary depending on the pulp stock used and the type of paper being made. However, certain general observations can be made, the understanding of which is essential to evaluate properly the merits of added sizes: (*a*) The strength developed in the paper sheet depends on the extent of beating. Normally, the strength slowly increases with the amount of beating up to a maximum. After many hours of beating a pulp suitable for making paper referred to as "glassine" is finally obtained. (*b*) The ability of the pulp to retain water when passed over the "wire" (as noted by allowing the suspension of pulp to settle in a tall glass cylinder) is increased to a maximum, depending on the extent of beating. This water-retaining ability is referred to as hydration, although it is doubtful that a cellulose hydrate is formed. It might be concluded that the strength developed in the paper depends on the extent to which the pulp is hydrated. (*c*) It follows that in the use of undried pulp in the beaters, that is, in a process in which manufacturing operations are continuous, less beating action is required to secure optimal results than in a process in which a dry pulp is used to supply the beaters. (*d*) Different types of pulp stock may require different degrees of beating to bring them to their respective optimal states of hydration. This difference is due to the source of the fiber, the extent of chemical digestion, and other variables in pulp-making. Other generalities will be given after a discussion of the added size.

The function of a starch size added at the beaters is primarily, as intimated above, to increase the strength of the sheet. Depending on the nature of the sheet to be made, less important functions are those to aid in making the sheet more impervious, to lay fuzz, to impart a crackle to the finished sheet, and to aid in making the sheet more resistant to such wearing actions as scuffing and folding.

It will be seen that a suitable sizing agent is one which is colloidally dispersed sufficiently to enable it to form a bond between the individual pulp fibers, and yet is not degraded to the extent that its adhesive strength is low or that it has become so water-soluble or dispersed as not to be retained by the fibers but rather will pass out with the water phase when the pulp is filtered on the "wire." Considerable effort has been spent in research directed toward the attainment of an optimal balance between these factors. As a consequence, starch has been used for sizing in the beater in a great variety of forms and states of pretreatment.

Raw, or ungelatinized, native starch has been added at the beaters, either owing to a lack of appreciation of the above considerations or to the possible supposition that if the starch were gelatinized, or solubilized, it would more readily pass into solution in the water phase and as a consequence less starch would be retained by the pulp. When raw starch is used, it is expected that sufficient heat and water will be available to gelatinize the added raw starch, which is mechanically held by the paper pulp, when the newly formed, wet sheet is subsequently passed over heated rolls to dry the paper. Raw starch has so litle adsorptive affinity for most types of paper fibers, however, that of the 1 to 3% starch normally added to pulp in beater sizing only 30% ot this starch is retained by the fiber. The amount that is retained, if examined in the finished sheet after being stained with iodine, will be seen to be very poorly dispersed (1). That is, the heat and moisture available at the drying rolls are not sufficient to gelatinize completely most raw starches, *e.g.* corn starch, and hence only a fraction of the starch retained becomes effective in creating a bond between the paper fibers.

To secure better efficiency in the use of an ungelatinized starch, it may be modified in manufacture to reduce its gelatinization temperature. For example, Casey (2) has shown the increased effect on the Mullen test for tensile strength, fold, and ink resistance tests secured (over unmodified starch) by oxidizing the starch before adding it to the beaters. The extent to which the starch is modified by oxidation, as judged by paste viscosity tests, does not significantly alter its efficiency, except in ink resistance. This result is in contrast to some of the other types of starch modification such as acid hydrolysis.

The next logical step in the efficient utilization of the starch is to cook the starch before adding it to the beaters. This secures greater colloidal dispersion with the result that, instead of a granule of starch bonding two fibers at only one point, the adsorbed or absorbed starch paste will be spread over a greater area and hence bonding between fibers will be secured (by the one original granule, for example) at many points. The underlying difficulty in this procedure is that, whereas raw starch is surprisingly resistant to mechanical treatment (such as to the disintegration applied in the beaters but more particularly in the Jordan disintegrators which frequently are used after the beaters), gelatinized or partially gelatinized starches, particularly the non-cereal starches, are very unstable to the action of physical forces. The result is that the degree of colloidal dispersibility of the gelatinized starch does not remain constant, but rather the starch continues to disintegrate and a large part of the starch is broken down to such an extent that it is lost at the "wire" (3). The extent to which starch should be cooked becomes, therefore, a matter of judgment and is conditioned by the type of raw starch used, by the differences between individual batches of any particular starch, by the pH of the available water, by the variation in the operation of the beaters and Jordan disintegrators, and so forth.

Methods have been proposed to circumvent the above difficulties. One method is to stabilize the cooked starch. Rowland (3), for example, cooked raw starch in the presence of formaldehyde and acetic acid and claimed that he secured the desired toughening action on the granules. A modification of the procedure is to pretreat the starch with formaldehyde and then disperse it by cooking it with sodium hydroxide before it is added to the beaters. This dispersed starch is precipitated on the fibers by the addition of acidic material, e.g. alum.

In respect to the latter procedure, other proposals have been made to secure better retention of rather highly dispersed starch on the paper fibers. Precipitants such as alumina have been recommended for this purpose (4). Kesler and Black (5) proposed that fatty material, *e.g.* fatty acids or soaps, be added, since they believed that a complex formation occurred with the dispersed starch. The complex is then precipitated on the fibers by the addition of metallic ions to form an insoluble metallic soap-starch precipitate. The fundamentals involved in the use of such precipitants on starch have been discussed by Heald (6).

The value of insolubilized starches, that is their bonding strength for cementing the various fibers together, is not very well known, as little has been reported concerning the relative increase in bursting, fold, tear, and scuff strength, permeability, etc., for a given addition of size.

It has been suggested that better efficiency will be obtained if the starch is chemically modified before or after the cooking process. For example, Cobb and coworkers (7) state that starch behaves like a highly hydrated cellulose. Very viscous starches, carrying relatively large amounts of water, when dried may induce an undue contraction in the sheet. If present in the amount required to bond all the fibers, the viscous starch may contract so much that air cells develop on drying and the starch films would not be continuous. These workers demonstrated that modification of tapioca starch, by enzymic action during the period of cooking, increases the drainage rate at the "wire," and improves the results with the Mullen and "pick" tests.

An additional objection to precooking the starch, raised by some of the larger mills, is that another operation is introduced into paper manufacture.

Starch millers and processors, working with the paper industry, have, therefore, sought to pretreat the starch and have experimented with methods to precook it so that the optimal benefits will be secured by adding to the beaters the dry products as supplied. The pretreated and precooked starches are prepared in the final dry form by passing the starch over heated rolls, by vacuum drying with the help of agitators, by spray drying, etc. A commonly used product is dried over hot rolls and is sold under the trade name "Amijel."

As reference to chapters on the physical and chemical properties of the starches will show, gelatinization of the starch induces a physical lability in the linear components of the starch, commonly called amylose. After the starch is solubilized in hot water, the amylose may irreversibly precipitate on aging and cooling. This phenomenon is often referred to as retrogradation. Once precipitated, amylose is not redispersed unless subjected to temperatures higher than 212°F. or to the action of alkali or some other chemical reagent. Hence it is impossible to obtain the equivalent of a freshly cooked starch paste with many of the pregelatinized products simply by the addition of water. A granular-appearing paste is obtained which, even after an extensive disintegration treatment in the beaters, is not sufficiently broken down to produce the desired dispersion. Although fair retention may be obtained with such a product, dispersion is poor, as reference to the data and Figs. 100 and 101 will show. The net result is poor over-all efficiency for such a product. Fig. 100 shows an iodine-stained sheet made by adding 2% of a roll-dried corn starch product to the pulp at the beaters. Obviously the extent to which a roll-dried product is ground after leaving the drying rolls will influence its dispersibility. Overgrinding may, however, reduce its retention in the paper fibre at the "wire." The particle size of the product becomes an important factor, consequently, in respect to its efficiency for beater sizing.

As mentioned before, alkalies and alkaline reagents tend to redisperse retrograded starch. Hence borax is incorporated in some pregelatinized starch products to facilitate redispersion. The borax serves an additional purpose in that it also acts as a wetting agent when the product is remoistened. This function will be mentioned again later.

Some starches retrograde less under the same conditions than others. Potato and tapioca starches show less inherent tendency to retrograde than certain cereal starches, as for example corn starch. Pregelatinized products made from the former starches, particularly if the product contains a small amount of dispersing agent, may be stirred up and beaten into a paste which approximates a freshly cooked potato or tapioca starch paste in colloidal properties. Properly used, such products have good dispersion in the beaters, good retention, and hence a relatively high efficiency as beater sizes. Dis-

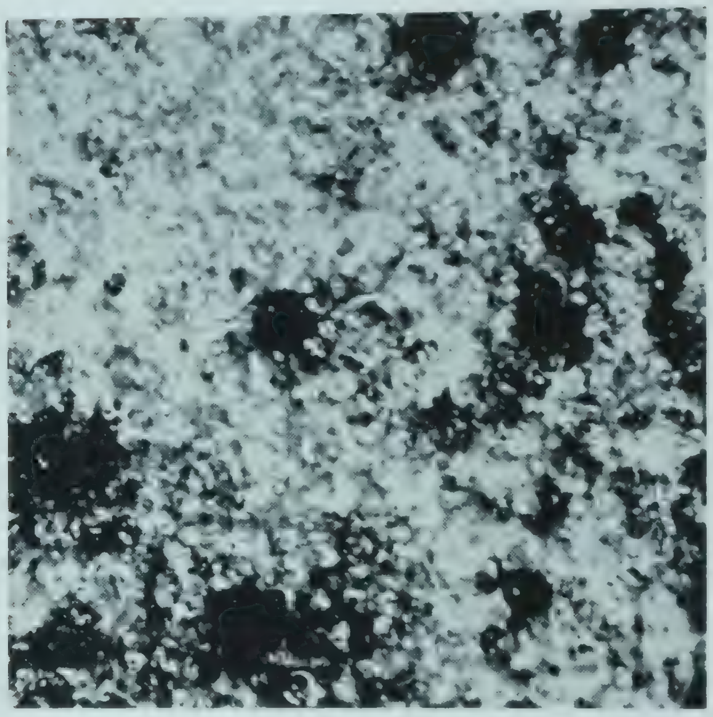


FIG. 100. Iodine stained sheet (× 100) sized with roll-dried corn starch. 80% retention. 7% increase in Mullen test.

advantages to be noted for these non-cereal products include the following. (a) Their dispersed pastes are relatively unstable to mechanical treatment. (b) They are made largely from imported starches. For this reason they are at times expensive or their supply is uncertain. Native potato starch is one of the most expensive domestic starches. (c) They wet rather poorly and tend to ball up when stirred into water. The particles take up water at such a rate that some form a gummy mucilage which then encases or surrounds many other dry starch particles. These larger aggregates beat out with difficulty.

In addition to having the inherent property of retrograding less, potato and tapioca starches are more viscous than corn starch. This latter property may be of aid in obtaining greater size retention for a satisfactory degree of dispersion.

One of the oldest and most commonly used methods for the industrial modification of corn starch to eliminate its retrograding tendencies is by oxidation, as for example with sodium hypochlorite. Simple oxidation for the purpose of minimizing this undesirable characteristic in a starch which is to be roll-dried, however, also results in such a physical degradation of the starch that the resulting product, when redispersed, is too low in viscosity for high efficiency as a beater size.

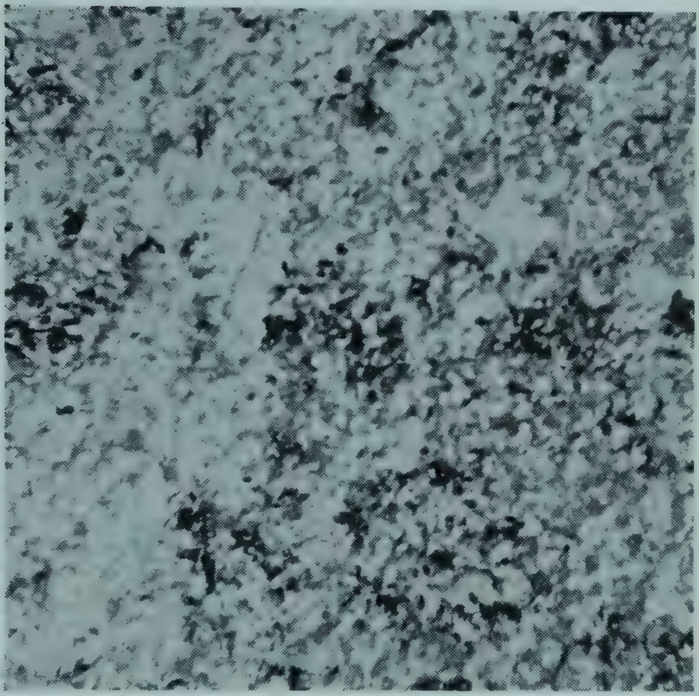


FIG. 101. Iodine stained sheet (× 100) sized with chlorine treated starch. 64% retention, 20% increase in Mullen test.

A method for pretreating a starch such as corn starch with chlorine which reduces the retrograding tendencies to such an extent that a roll-dried product may be made which redisperses in the beaters to a very satisfactory degree has been proposed by Kerr (8). At the same time so little degradation of the starch units accompanies this pretreatment that retention is not impaired. The result is an over-all efficiency for the product which compares very favorably with those made from potato and tapioca starches. The addition of borax to such a pretreated corn starch before it is passed over heated rolls, permits the gelatinized, dry product to wet uniformly (see Fig. 101). It may, therefore, be used with less difficulty than the non-cereal ones. Indeed so superior are its redispersing properties that it is found possible to add the size to the pulp after it has left the beaters (but before it has passed the Jordan disintegrators). The latter practice, if possible, is preferable in many paper mills, since the beater operation may take place an hour or more previous to the time when the final sheet is finished. Variation in the tensile strength of the paper is not as readily corrected by adding more or less starch if the adjustment must be made at the beaters. However, if the adjustment may be made at the Jordan disintegrators, variations in tensile strength may be corrected within a few minutes, and the amount of paper produced which does not satisfy the required specifications may accordingly be reduced.

The discussion above as to the dispersibility of the starch size refers primarily to treating paper of average density. Casey (9) has pointed out that a highly dispersed starch need not necessarily give the optimal increase in strength to a very low density paper. In such cases the paper fibers are in contact with each other at only a very limited number of points and there is apparently no reason to use a starch so highly dispersed that it will coat the entire surface of the fiber when a normal ratio of 2 to 3% of starch is added to the paper pulp. The conclusion presented was that poorly dispersed starch suspensions which are composed predominately of swollen starch granules are most effective in low density sheets and least effective in high density sheets. However, when a very dense sheet is being formed, the fibers are in contact with each other over a large portion of their surface and here it was found that highly dispersed starch solutions were most effective.

Recently, Kerr (10) has patented a method for treating starch with sodium stannate which highly disperses the starch into a solution of high viscosity. The addition of this solution to paper pulp very materially increases the bursting strength. It was reported that 200 lbs. of starch cooked with 4 lbs. of sodium stannate in 800 gals. of water and added at the head box to 13,333 lbs. of spruce pulp, increased the Mullen test 14.5%, whereas the same amount of starch cooked with 3 lbs. of NaOH only increased the Mullen test 4.4%. Alternately, the treated starch may be roll-dried and added as a powdered product at the beaters.

An approach to a fundamental study of the use of starch in paper sizing has recently been attempted by Kerr (11). It was pointed out that it has long been known from the work of Tanret (12), as well as that of Pacsu and Mullen (13), Samec (14), and others, that cellulose fibers selectively adsorb amylose (the linear component) from starch solutions. The "retention" of this component would accordingly be expected to be high. However, the amylose (at least one with the degree of linearity or of the length of chain present in corn amylose) orients with linear polymers such as itself or cellulose to an extent that the ability of this starch component to form a continuous bond from one fiber to the next is probably very slight. Stated otherwise it may be said that amylose shows a pronounced tendency to retrograde either with itself or with cellulose fibers and that such effects contribute to the production of less continuity in the starch film. On the other hand, the larger, branched configurations present in starch are adsorbed very poorly by cellulose. Their adhesive strength in this connection might not be expected to be of a high order but the amount retained by paper pulp should on drying, none the less, create a continuous bond between pulp fibers. This result has been confirmed in work by Kerr and Schink (15). When 2% of corn starch (based on the dry weight of pulp) is heat-gelatinized and added to a paper beater, it is found that the bursting strength (by Mullen's test) of the finished sheet increases 5% above the bursting strength determined on an unsized sheet. Two per cent corn amylose added in dilute, hot solution to the beater is adsorbed to the extent of 48%, almost the anticipated limit,* (16) but the bursting strength of the sheet is no better on the average than for the paper sheet without the added size. In contrast, the addition of 2% corn amylopectin to the beater results in an increase of 11% in the Mullen test value for bursting strength over that for the base pulp. The mechanical absorption of the amylopectin by the paper sheet is apparently increased over that anticipated by the addition of rosin and alum. These adjuncts were added, according to the usual practice of paper manufacture, in all of the tests reported. Under such conditions it is found that 60% of the amylopectin added is retained in the paper. Considering the fact that corn starch is about 70% amylopectin, it is difficult to conceive that the presence of amylose in whole starch pastes contributes to the effectiveness of the starch in respect to increasing the bursting strength of the sized paper. It might be supposed, however, that in mixtures of the two components, such as in native starch, the adsorption of the linear fraction on the paper fiber forms a base on which the amylopectin may more readily form a bond. The results with whole starch, however, do not support the view that this bond is stronger than the one created by the amylopectin itself when the sheet is dried.

Casey (9) has also found that dispersed, or non-dispersed corn amylopectin, depending on the density of the paper, has a greater effect on increasing the Mullen test than solutions of amylose, or dispersions of whole corn starch.

Fowler (17) has claimed that the wet tensile strength of the paper may be materially increased by dispersing both starch and rosin in sodium metasilicate solution, adding a condensation product of urea and formaldehyde followed by adding alum until the pH of the beater mix is brought within the range of 4.2 to 5.8, preferably between pH 4.6 and 4.8. By using a proportion of 40 lbs. of starch, 20 lbs. of rosin, 30 lbs. of sodium metasilicate, pentahydrate and 57 lbs. of the urea-formaldehyde product to 1400 lbs. of paper pulp, the wet strength, with the machine, was increased 60%, and cross machine, 80%. In addition, the tear and fold tests were very materially increased.

3. **Calender Sizing.** As the name implies, calender sizing is accomplished by the addition of size solution to one of the water boxes on the calender stacks. That is, the web of the sheet has already been formed but the

---

*Pacsu has indicated, and Kerr and Severson (16) have confirmed the result that cellulose fibers will adsorb up to 1% of their weight of corn amylose.

sheet has not been thoroughly dried. Starches, or starch products, are used almost exclusively for this operation. It is apparent from the manner in which the starch is applied and from the characteristics of the size that the sizing is almost purely a surface addition. Calender sizing finds its greatest application in paper board mills, and it is used to improve the surface of the sheet and to lay loose fuzz. All types of starch are utilized; *i.e.*, from native or unmodified starch to relatively thin boiling modifications. The choice of starch is guided by the results desired. The final results depend principally on the amount of starch applied to the sheet, which is related primarily to the content of dry substance in the size. The viscosity of the size is more or less fixed within limits by the mechanics of the sizing operation. Generally the less the starch is modified, the less will be the dry substance present in a size at a given viscosity, and as a result the smaller will be the amount of starch added to the sheet. On the other hand, the use of highly modified starches will result in a substantially larger amount of starch added to the sheet than results when native starch is used. A secondary objective to be accomplished in calender sizing is, as intimated above, to lay surface fibers. They should not only be laid to form a smooth, attractive surface as the sheet leaves the calender rolls but these fibers should resist a certain amount of scuffing action after the board has been put into use by the purchaser. In general, the more a starch is modified to reduce its paste viscosity, the less will be the resistance to scuff of the board sized with the modified starch. As a result, a balance is sought between the weight of size added to the paper and scuff resistance and this is accomplished by selecting a starch modified to some intermediate level.

Other effects can be obtained by calender sizing. Some paper-board makers desire to prepare a board with a surface which will be suitable for printing with gloss ink. This can be accomplished by the use of a highly modified starch so that a relatively high concentration of the starch can be used for the size (for example, 1½ to 2 lbs. per gallon). The use of starches of high fluidity results also in a penetration of some of the starch beyond the surface of the sheet. In such cases an increase in the bursting strength of the sheet is obtained.

From the nature of the paper product treated by a calender size operation it is obvious that the more expensive types of modified starches, such as oxidized starches and dextrins, are rarely employed. In order to obtain paper surfaces more suitable for some uses or to obtain special effects in finishing, other ingredients may be added to the starch size; for example, wax emulsions. Some of the special starches for calender sizing are prepared by the starch manufacturer by the inclusion of suitable waxes or similar ingredients.

4. Tub Sizing. The fundamentals of starch chemistry as applied to paper sizing have been discussed by Kerr (11) with particular reference to tub sizing. Since in this application the preformed sheet is immersed in a dilute size solution of low viscosity, it is desirable that the starch product be pretreated to reduce its paste body to the level at which the size will penetrate the sheet to the desired extent and will add dry substance to the finished sheet within the limits of weight desired. Reduction in paste viscosity can be obtained in several ways. The more frequently used methods are by the use of acids, oxidizing agents, conversion by enzymes, and dextrinization by heat. For the purpose of tub sizing paper, it is obvious that a method for modification should be selected which will either tend permanently to solubilize the amylose or will tend to degrade it preferentially, leaving the amylopectin in a high state or degree of polymerization. Incorrectly modified starches, in particular corn starch, are very readily recognized by the poor results obtained in practice from such poorly modified products. The viscosity of the size will increase on standing or it will increase during the period of time the size is used. An increase in the viscosity of the size may result in less penetration and an increased weight of starch applied to the paper, usually on or near the surface of the sheet. Tub sizes are normally made by diluting starch pastes to a relatively low concentration of solids. The amylose, if insufficiently modified, becomes unstable in colloidal solution when the paste is diluted and it tends to separate as an insoluble phase. From these sizes the liquid phase is preferentially absorbed by the dry paper sheet and the insoluble material is continuously returned to the size box by the action of the squeezer rolls through which the paper sheet passes after leaving the size box. The effect increases the consistency of the size. Eventually, the insoluble material tends to cohere and lumps become mechanically enmeshed in the paper fiber. Very uneven sizing results, giving the paper an unattractive appearance.

Animal glue has been and is being used to tub size paper. Some paper manufacturers, especially those making certain types of white writing paper, prefer to use mixtures of glue and a starch product in order to secure the beneficial results of each. Glue or certain constitutents of a glue size are not compatible with solutions of undegraded amylose. Even in dilute solutions, the stability of the amylose is reduced in such mixtures by the presence of the glue size. When some glue is employed, it becomes increasingly important, therefore, that the amylose component of the starch be degraded or solubilized to such an extent that it will not retrograde.

Oxidized starches have been used to good advantage in tub sizing. The stability of oxidized corn starches is well known. However, their price is relatively high compared to other modified starches and to some of the other sizing agents available.

Very good results have been obtained in recent years when a properly prepared torrefaction dextrin was used. The proportion of linear chained polymers in some of these dextrins is small, as judged by butanol precipitation. (11). The results obtained with dextrins of this type are in harmony with the theories advanced by Brimhall (18) that in the process of dextrinization by heat it is possible to join the linear polymers by new glucoside linkages to form branched configurations.

Principally for reasons of economy in recent years there has been a most remarkable increase in the amount of starch used by the paper manufacturer for tub sizing that has been converted with enzymes. This modification and application of starch is discussed elsewhere.* The success of the method in this instance depends upon selecting the proper conditions to direct the enzymic action so that in the liquefaction of the starch to the desired viscosity, the amylose will not be left in a state in which it will interfere with the paper-sizing operations or results. In this connection the work reported by Kerr *et al.* (19) should be noted. Technologists of the paper, enzyme, and starch-milling† industries have cooperated in this development with the result that the paper manufacturer already has at his disposal methods for making acceptable tub sizes by the use of enzymes and of native starch. Judging by the rate at which these developments have been observed in practice, it may be concluded that ideal tub sizes may soon be produced by such means.

5. **Surface Sizing: Clay Coatings.** Certain types of paper are surface-coated with clays or similar fillers. The size may also contain colored pigments, water-proofing agents, or other substances to give the desired weight, color, and finish to the sheet. For example, the better grade of magazine or periodical paper is surface-coated with a clay mixture which, after being dried and ironed over calendar rolls, takes on a glossy surface which is relatively impervious to printers' ink. Naturally, better printing is possible and sharper photographic reproductions are obtainable. Many other diverse types of coatings besides clay coatings could be listed. The art of paper coating is very complex indeed, and it is beyond the scope of this chapter to discuss all types. Only those which involve the largest outlet for starch products will be treated. Coating may be applied to paper by any one of several methods, and the discussion is divided according to the method of applying the coating, since the process which is employed in a large measure determines the characteristics required in the coating.

---

*See Chapter XVI.

†See, for example, the recent report of Gillan (20).

The older methods involve passing the predried paper sheet between applicator rolls which apply a given amount of size to the paper. The paper then passes through a series of brushes which spread the coating evenly over the paper surface. The paper is dried by passage through a heated chamber and is calendered. Both sides of the paper are usually coated in the same operation. In one modification of the above process highly polished metal rolls are used instead of brushes to spread the coating. Another modification is the use of a fine jet of air at high velocity to spread the coating and to remove the excess which then runs back to the size box. Although a relatively high speed may be maintained on such machines, difficulties are encountered when attempts are made to coat both sides of the paper simultaneously. The above types of coatings, as a class, are referred to as "brush coatings" and the characteristics required in the coating are very nearly the same for each method mentioned. The principal determining factor is that operating conditions for this type of work usually require that the coating contain not more than 55 to 60% of water. The use of more water frequently results in a failure to put the required weight of clay on the paper, and in addition requires more heat, or drying time, for drying the coated paper than when smaller amounts of water are used.

However, clay mixed with an equal weight of water is an unworkable mud. Even if it were thinned to a usable consistency, it would not adhere the paper fibers when the paper is dried. Hence, wetting agents, protective colloids, and adhesives are added to the clay to secure the proper colloidal characteristics. Casein, cooked in alkaline media, is very nearly an ideal colloid for this purpose. The addition of 5 to 10% of casein reduces a clay mud to a mobile liquid which may be evenly brushed over the surface of the paper. The casein has, furthermore, a high adhesive strength in binding the clay to the paper fiber. It is, however, even in normal times, a relatively expensive product to use in the manufacture of paper. Starches have therefore been modified to replace casein. The advantages to be listed for the use of starch as compared to the use of casein are that the starch is free from odor, is non-nitrogenous, and possesses fewer inherent foaming tendencies. Being non-nitrogenous, it is less susceptible to decay and, hence, the starch-coated papers possess greater permanence. Excessive foaming is to be avoided in coatings, since froth tends to create a pitted surface on the sheet. To secure the desired adhesive strength, more starch is required, pound for pound, than casein. For this reason and also in order to develop greater protective colloidal properties, the raw starch is modified. This modification includes a reduction in paste viscosity so that the final starch-clay mixture will not be thickened because of the added starch. The most common modification of starch which accomplishes the results described is chlorination. Highly chlorinated starches are used in a ratio of about 15% starch to clay. The resulting coated sheets are similar in many respects to those sized with casein-clay coatings. The starch-clay coating does not possess quite the adhesive strength or the water resistance of the casein-clay coating. Water-resistant surfaces are desired for certain papers. Naturally, better bonding between clay and paper fiber results by adding a higher ratio of starch to clay. Two undesirable effects are noted as a result of this practice: a duller sheet is obtained, that is there is a loss of gloss, and, secondly, the viscosity and the plasticity of the coating are increased with the result that it becomes more difficult to spread the coating during the paper-sizing operation. To correct this effect either more water may be added to the size, which addition slows the production rate in the drying operation, or a starch that is more highly modified and of thinner viscosity may be employed. Experience has shown, however, that if starch is modified beyond a certain point degradation reactions then predominate to the extent that the adhesive strength and the protective colloid effect of the starch are reduced at a more rapid rate than the viscosity and plasticity of the starch are reduced.

No doubt much of the unfavorable reputation established for starch in paper coating can be traced to improper application due to a lack of appreciation of the characteristics of starch, outlined above. Another factor contributing to the improper use of starch is that a sol, prepared by cooking a highly chlorinated starch, is a relatively labile, colloidal system. Excessive cooking of the starch, or excessive heat at any point in the operations which follow, tends to degrade or destroy the colloidal properties of the starch. Excessive mechanical action, such as occurs in mixing the clay, starch, and water as well as the agitation of the size in the circulation pumps and at the coating machines, tends to thin and weaken the starch. The working characteristics of a batch of clay and starch may, therefore, tend to change during the period of its use. When poor results start to develop, as noted first by a decrease in the viscosity of the size, then by poor binding action of the starch in holding the clay to the paper fiber, the natural tendency of the operator is to add more starch to the unused portion of the batch of size. An increase in plasticity is often noted as a result of such corrective procedure by the development of streaks in the sized paper due to poor brushing. If more water is added to secure smoother brushing, the size not only requires more time or heat for drying but also becomes weak in adhesive strength.

In normal periods, the difference in price between the so called chlorinated starches, or gums, and casein hardly compensates for the variation in the grade of coated paper produced. This variation results from a lack of appreciation of the behavior of chlorinated starches, a failure to exercise proper control in their use, or a combination of these factors. In recent years, starch chemists, working with paper chemists, have devoted considerable effort to finding improved methods for modifying starch and for applying the starch product. It should be noted before passing on to these developments that the starch chemist has, in the meantime, made considerable improvement in the colloidal stability of chlorinated starch. Several brands are now on the market which are decidedly improved compared to those formerly marketed.

With chlorinated corn starch as a reference or standard for the comparison of starch products in clay coatings, other means of modifying the starch will be discussed, and the resulting products will be compared with the standard in an attempt to develop certain general principles.

If corn starch is modified by an acid treatment to such a degree that the hot paste viscosity of the product is similar to that of the chlorinated starch referred to above, and a clay coating is made with this acid-modified starch, it will be observed that the coating is more plastic in nature and that the adhesive bond formed between clay and fiber is weaker than in a similarly prepared coating in which chlorinated starch is used. To explain the plasticity of the coating, it may be noted that the acid-converted starch tends to gel or retrograde more after cooking and cooling than the chlorinated starch and that its protective colloid effect on the clay is less. The action of acid in reducing the paste viscosity of a starch is accomplished primarily by a scission of glucoside bonds between the glucose units which make up the various starch molecules. These ruptures weaken the physical structure of the starch granule and thereby reduce its paste viscosity. As the action is extended, there is a decided decrease in the average molecular magnitude of the constituents of the granule and there is a parallel loss in the adhesive strength of the modified starch. It is to be noted that in the intermediate stages of the hydrolysis the percentage weight of starch precipitable by butanol increases to a value above that for untreated starch. Hence it must be assumed that before a material degradation in the linear chain components of starch is accomplished by the acid, the branched components suffer a decided decrease in their molecular magnitude (breaking apart in fragments) to form additional quantities of linear polymers. Chlorination, on the other hand, reduces the paste viscosity of a starch without reducing (actually increasing) the colloidal stability and the protective colloid effect of the starch, both of which properties are usually associated with the amylopectin fraction. The percentage weight of starch precipitable by butanol gradually decreases during an oxidation of starch with chlorine.

From the foregoing discussion it might be presumed that the ideal starch product to use in preparing a clay coating would be made by destroying the granule structure by extensive gelatinization, either by cooking at high temperature or by solution in alkali and thereafter freeing the amylopectin from the amylose. This would seem to follow from the result that the isolated amylose fraction possesses a viscosity that increases abnormally with an increase in concentration or with a decrease in the temperature of solution; also, that its solutions show exaggerated tendencies to gel and that it possesses, if any, a negative protective colloid effect on clay suspensions. The author has performed such a separation using butanol to precipitate the linear polymers from the amylopectin and has attempted to prepare coatings from both of the separate fractions (21). The results of this experiment disclose that the amylopectin is too viscous to be used as such in clay coatings if the usual ratio of about 15% of starch product to clay is employed. The use of a reduced concentration of amylopectin in order to obtain a clay coating of workable viscosity results in a coating on paper with an adhesive strength below that desired; that is, the clay is not sufficiently bound to the paper fiber after the coating is applied and dried. Some degradation of the amylopectin fraction is therefore required. As might be anticipated, the complementary fraction of the starch, the amylose, could be studied in clay coatings only when a low concentration of the product was used and when temperatures above 70°C. were employed for application of the coatings. With these experimental conditions, the coating could be evaluated and it was found that the amylose-clay coating proved to have a higher adhesive strength than amylopectin at a like concentration. Either an increase in the amylose concentration to obtain a coated paper suitable for printing or an attempt to apply the coating to paper at a temperature normally used in practice results in an unworkable plastic mass of clay which very soon gels. Thus, although native amylose possesses better adhesive strength in this application than native amylopectin, the latter has all of the other colloidal characteristics desirable in an adhesive-carrier for clays except that in its native state its viscosity is too high. Since some degradation or modification of the amylopectin fraction is desirable, the possibility of starting with whole starch and designing a modification which will preferentially degrade the linear chains at a greater rate, or which will alter the colloidal characteristics of the linear chains in a desirable fashion during the modification, is worth considering.*

---

*The work of Diehm (22) suggests that the highest clay-binding power of a starch product results when the greatest reduction in viscosity is obtained for least reduction in molecular magnitude. Considering the abnormal viscosity effects produced by amylose and that the latter contributes little to the average D.P. value for starch, this view can be harmonized with the one presented above.

The use of enzymes to modify the starch for use in clay coatings has been studied. Enzymes are selective in their action in respect to the chemical configuration of the substrate. It is known that the linear components of starch are readily hydrolyzed to maltose by distase, particularly by the β-amylase constituent of commercial distases. The branched configurations present barriers to an extensive degradation by the saccharogenic factor, β-amylase. It seems possible that by selecting the proper combination of amylases and by adjusting conditions for conversion it would be possible preferentially to modify the less desirable amylose constituent. At the same time it seems possible to eliminate the effect of the granule structure of the starch on the paste viscosity and to reduce the viscosity of the resulting colloidal solution of amylopectin to the desired value. Several problems are involved in the use of this method, a solution for which is not readily apparent. β-Amylase speedily and completely degrades the linear glucopyranose chains which are available by producing sugars such as maltose. Since sugars have low adhesive strength and poor colloidal properties, that part of the amylose split by the enzyme to sugars is a loss as far as coating properties are concerned. Furthermore, not all of the linear polymers in starch are readily available to the enzyme. Some part of this fraction is apparently in a physical state which makes it almost invulnerable to attack by the enzyme.

The use of commercial enzyme preparations which contain a predominant amount of α-amylase may lead to the production from amylose of dextrinous bodies which will retain a sufficient length of chain to contribute to the adhesive strength of the converted starch. Nevertheless, it is likely that a share of the amylopectin also will be reduced materially in molecular size in the presence of this enzyme by the more random splitting of glucosidic linkages. That is, the hydrolysis with α-amylase appears to lead to results similar in some respects to those obtained by acid hydrolysis.

To overcome one of the major objections to enzymic modifications given above, Kerr and coworkers (19) have suggested that the starch processor might pretreat the native starch with enzymes, using such conditions that a considerable portion of the linear components of starch is removed as sugars of low molecular weight. These sugars can be removed by washing and recovered to be sold for some suitable use. The residual starch can be additionally modified with enzyme by the paper mill technologist to produce a superior starch and claycoating mixture.

Considerable research by paper technologists is in progress on methods for the preparation of superior coatings in which starch is used that has been modified by a single treatment with enzymes at the paper mill. In general, sizes made from enzyme-converted starches, such as corn starch, show a measurably more plastic character than similar sizes prepared from chlorinated starch. However, a very significant contribution has been made by the paper chemist in recent years which not only permits the use in clay coatings of such enzyme modifications but of other types of modified starches, the use of which will be discussed.

It has been known that a part of the plasticity of a clay coating is due to the presence of the clay itself. The relative proportion of the plasticity of the coating for which the clay is responsible has not been appreciated until recently. Formerly, the clay was wet, or fluxed, by the addition of sodium carbonate, borax, or some other mild alkaline reagent to the clay and water mixture. The added adhesive was not only expected to develop no plasticity itself but also was expected to disperse or colloidally protect the clay in the coating mixture. It has recently been found that the addition of very small amounts of such alkaline wetting agents as tetrasodium pyrophosphate to almost solid masses consisting of 2 parts of clay to 1 part of water (by weight) reduces these mixtures instantly to free flowing suspensions. The function of the added colloid then becomes principally one of supplying adhesive strength for bonding the clay to the paper fiber and, secondarily of supplying whatever protective colloid action is required to keep the clay evenly dispersed while it is being applied. This latter requirement is small because the agitation, which is present in many sizing processes, is in itself sufficient to prevent the clay from sedimenting. The more recent practice is, accordingly, to flux the clay with water in a ratio of about 67% clay to 33% water (by weight) by adding approximately 0.2% tetrasodium pyrophosphate (based on the dry weight of clay present). Clays differ in their requirement of pyrophosphate for fluxing. It should also be noted that the inclusion of other pigments in the coating may increase the amount of flux required. For example, free lime in calcium carbonate, a pigment which is frequently mixed with clay, substantially increases the amount of pyrophosphate required to obtain the maximum fluidity, or fluxing action. The adhesive is prepared at a suitable concentration and added to the fluxed clay in a high speed mixer, ball mill, or mixing equipment. Usually, starch is cooked at a concentration of about 20% with water and added to the fluxed clay in the proportion of about 15 to 20 parts of starch (dry basis) to 100 parts of clay. Only a few minutes of agitation are normally required to secure an intimately mixed clay coating, whereas formerly a considerably longer period of ball milling was required when less efficient fluxes were available.

The use of more suitable fluxing agents has not only permitted the use of dextrinous products arising from the treatment of starch with amyloclastic enzymes, but has also permitted the use of other modifications of starch with high adhesive strength. Certain types of torrefaction dextrins have been employed (21). Still other modifications have been used in which the starch is reduced in viscosity, probably more by a destruction of the supermolecular forces between the units of the starch than by rupture of primary valences within these units. An example of this type of modification is, possibly, the action of relatively high concentrations of strong organic acids, such as acetic acid, on starch. Oxidized starches, other than a chlorinated type have also been used in clay coatings; for example, "peroxidized" corn starch.

TABLE XXXII

Characteristics of Starch-Clay Coatings

15 parts of starch to 100 parts of clay; 45% of total solids.

Type of corn starch used as adhesive	MacMichael viscosity	MacMichael yield value	Dennison wax test
Chlorinated	30.5	7.6	3.25
Peroxidized	28.2	1.0	4.50
Special torrefaction, Dextrin A	28.5	2.5	4.50
Native starch, enzyme-converted	32.0	3.0	3.75
Residue starch, enzyme-converted	29.0	0.0	4.50
Acetic acid-treated	31.0	0.0	3.50

The characteristics of clay coatings containing several of the starch modifications mentioned above are given for comparison in the following discussion. The method for preparing the coating and for evaluating its characteristics is essentially as follows: The starch is gelatinized by heating a 20% suspension (by weight) in water to 95°C., with stirring, and maintaining the starch paste at this temperature for 5 minutes. The paste is cooled to 37°C. and added to a sample of domestic clay which has been fluxed with 0.2% tetrasodium pyrophosphate in water, a ratio of 2 parts of clay to 1 part of water (by weight) being used. High speed stirring for 10 minutes at room temperature is used to obtain intimate mixing. These conditions are satisfactory for the starches listed in Table XXXII. When modification of starch by enzyme was employed, the cooking procedure was altered by including in the mixture 0.85% of a commercial liquefying enzyme known as Amyliq, adjusting the pH of the suspension to 7.0, and maintaining the temperature of the starch slurry at 77°C. for 30 minutes, with stirring, before bringing it up to 95°C. for the 5 minute cooking period.

The viscosity and plasticity* of the coatings are measured directly after the coatings are mixed, in a MacMichael viscosimeter at 25°C. A No. 2 torsion wire connected to the disk type of plunger is used, and the torqu that develops is noted in degrees MacMichael as the viscosity cup containin the coating is allowed to revolve at several different speeds. When the degree of torque are plotted against r.p.m., a straight line is obtained if the coatin has been properly prepared. Exceptions are noted when starch products wit inherently high viscosities or abnormal tendencies to gel are used or i extreme limits of speed (r.p.m.) are employed. Since viscosity may be defined as the shear for a given rate of shear, the slope of the curve for a flui is proportional to its viscosity. A speed of 56 r.p.m. was arbitrarily selecte as a unit rate. The MacMichael viscosity then becomes equal to the degree estimated at this speed. Several typical curves are shown in Fig. 102. I will be noted from the curves that, in general, clay coatings are not tru fluids, since, when their viscosity curves are extrapolated to zero r.p.m., the lines do not pass through the origin. Most coatings exhibit plasticity, which may be estimated from the point of interception of the extrapolated viscosity curve with the axis for degrees MacMichael. This point, when expressed as degrees MacMichael at zero r.p.m., is taken as an index of plasticity of the clay coating and is called the yield value.

---

*The writer is indebted to Dr. F. Frost for the methods suggested to evaluate the viscosity and plasticity of clay coatings.

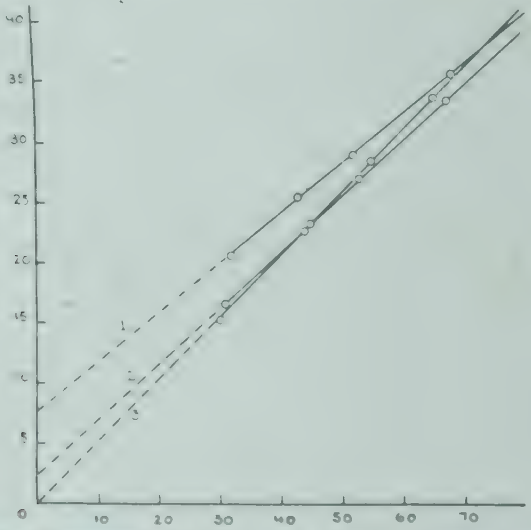


FIG. 102. Viscosity characteristics of starch-clay coating mixtures. Curve 1, chlorinated corn starch Curve 2, "peroxidized" corn starch; Curve 3, modified, residual starch from a diastase treatment at low temperature. Abscissa, r.p.m. of the MacMichael viscosimeter; ordinate, degrees MacMichael.

A stock of unsized, bleached sulfite paper was procured, and sheets were coated with a common type of laboratory applicator. After the coated sheet was dried and conditioned, the adhesive strength of the coating was evaluated by the Dennison wax test at a fixed temperature and humidity. Th test, which involves the application to the coated paper of melted, hot waxes, which are graded by their degree of tack, is frequently used by the paper industry to predict whether the coating will be strong enough, when printed, to withstand the tack of the printing ink impressed on the paper with type or other impressions. The results of the test are expressed on an arbitrary scale, and the higher the number of the wax, the greater is its tack. The values given in Tables XXXII and XXXIII denote that the coated papers used in the tests showed no removal ("pick") of coating from the paper fiber when a Dennison wax corresponding to the number listed was melted, applied to the paper, and pulled away, standard conditions for the test being used. The fractional values given in some instances indicate the proportion of all duplicate tests that showed no "pick" when the next higher wax in the Dennison series was used. Thus a wax test of 4.5 indicates that in all tests of the coating there was no "pick" on No. 4 wax and in half of the tests no "pick" on No. 5 wax. On this scale of values, a wax test of 4 indicates that satisfactory printing should result from using the coated paper with ink of average degree of tack. A wax test of 3 or lower indicates a weak coating and a wax test of 5 or higher, an excellent coating.*

All of the coatings listed in Table XXXII were made with 15 parts of starch (dry substance) to 100 parts of clay and a total dry substance of 45% in the coating. These values were adjusted before the final mixing operation by addition of appropriate amounts of starch sol and water. The coatings were all of approximately the same viscosity. A viscosity range of 25 to 35 MacMichael degrees is suitable for brush coating work. The relative plasticity of these coatings, as indicated by the yield values, showed considerable variation. Coatings with yield values above 10 are almost incapable of being spread to make an even coating on the paper by the applicator, whether it is a brush, a metal roll, or an air knife. A yield value between 5 and 10 indicates a passable coating and a yield value below 5 indicates excellent spreading quality. It is to be noted that the coating made with the chlorinated starch showed the highest degree of plasticity when the formulation and method for preparation described above were used. This is surprising, since the starch sol itself, at the concentration used, exhibits the least plasticity of all the starches tested. This might indicate that the fluxing action of the pyrophosphate and of the chlorinated starch is not an additive action. A similar result is obtained when one attempts to use mixtures of starch and certain proteins as suspension agents for clay. The combined

---

*For additional methods used in evaluating a starch for clay-coating paper, reference is made to the work of Saxl (23). The TAPPI tentative standard for the wax test is given (*Paper Trade J.* (TAPPI), **115**, 220 (1942)).

effect is not equal to the sum of their independent effects on clay suspensions. The modified, residual starch ("Residue starch"), which was pretreated with diastase at a low temperature to remove those elements of the starch that contribute most to the plasticity of its paste, makes a clay coating mixture having a mininum of plasticity, as might be anticipated. In addition, this coating exhibits about the maximal adhesive strength of the samples tested. The coating made from starch pretreated with acetic acid also exhibits a minimal plasticity. The action of the acid in the pretreatment is difficult to explain and is probably quite complex. From the results obtained it might be assumed that the crystalline regions of the original granule have been solubilized and that the component molecules have been so modified that their tendency to reorient has been greatly reduced. It is also to be noted that both "peroxidized" corn starch and the special torrefaction dextrin of corn can be used to make acceptable brush coatings by the formulation suggested.

The benefits to be derived from completely dispersing the starch for use in clay coatings has been pointed out by Casey (25). In general, the more the starch is dispersed into smaller colloidal units, the smoother is the surface of the uncalendered, coated sheet.

The second, and more recently developed method for the application of coatings to paper is the so-called, machine coating process. An essential difference between this method and the one previously discussed is that whereas brush coatings are applied in a separate operation to a dried sheet of paper, machine coatings are applied to the web of a sheet on the paper making machine by the use of specially designed applicators introduced, usually, in the press or drier sections of the machine. An outline of one method has been given in some detail (26). A summary of the development of machine coating has been presented by Massey (27), a pioneer worker in this field. Haywood (28) has recently given an excellent discussion of the technology of machine coatings using starch as the adhesive.

Because of the rate, in linear feet per minute, which paper travels on the more modern paper machines, as well as for other reasons, it is desirable that machine coatings contain a high solids content, that is, contain a small proportion of water so that the added drying operation can be accomplished in a short time without unduly increasing the number or size of the drying stacks. Therefore, the primary distinction between brush, and machine coatings is that the latter contains a higher content of solids, usually of the order of 50 to 65%. Other differences will be apparent from the following discussion.

The reduction of the water content of machine coatings to the level indicated requires that a much smaller amount of water may be used for the preparation of the adhesive than is customary in the preparation of the adhesive for brush coatings. Otherwise, insufficient water is available properly to preflux the clay. Hence for machine coatings, highly modified starch products with very low viscosities are required. They should be so modified that they may be cooked to fluid sols with about 2 parts of water. This requirement would seem to place a limitation on the use of many starch modifications since it is known that when starches are modified beyond the level necessary for the production of a satisfactory brush coating, thereafter in many types of starch modifications there is a sudden and decided decrease in the adhesive strength of the product. However, as the density of coatings is increased, another principle relative to adhesive value becomes operative, which has not been discussed. An experiment will now be given to show that the same starch, and in the same ratio of starch to clay, imparts greater adhesive value to the coating as the total solids content of the color increases, and, as a consequence, a starch product which may be weak in a coating of low solids content, actually may be quite satisfactory in a high density coating.

The "peroxidized" corn starch mentioned in the preceding paragraphs was used to make three different coatings varying in total dry substance content from 35 to 45%. Exactly the same proportion of 15 parts of starch to 100 parts of clay was used in each case. The coatings were prepared and tested by the methods previously outlined and the results are summarized in Table XXXIII. This experiment would seem to indicate that as the content of dry solids of the coating is increased the strength of a given adhesive is increased. Stated differently, as water is added to a coating more adhesive is required and conversely as the content of water becomes less, less adhesive is required. Therefore, it seems probable that, if the total solids of the clay coating were increased to 55 to 60%, a satisfactory coating composition

TABLE XXXIII

Characteristics of Starch-Clay Coatings Varying in Composition

Type of corn starch used as adhesive	Ratio of starch to 100 parts clay	Per cent total solids	MacMichael viscosity	MacMichael yield value	Dennison wax test
Peroxidized	15	35.0	10.0	0.0	3.50
Peroxidized	15	40.0	15.5	0.3	4.00
Peroxidized	15	45.0	30.0	2.5	4.50
Special torrefaction, Dextrin B	12	57.5	39.0	7.0	4.00
Special torrefaction, Dextrin B	10	57.5	30.0	3.0	2.00

with only fair intrinsic adhesive properties could be prepared by using a materially reduced ratio of starch product. This probability assumes that the clay will be properly prefluxed with a very efficient agent, such as tetrasodium pyrophosphate. These developments have suggested that a slight alteration in present methods for producing corn torrefaction dextrins, by reducing the paste viscosity under conditions of both a minimal hydrolytic degradation and color formation, would lead to a satisfactory adhesive for this type of clay coating. This type of modification was used for the preparation of the special corn Dextrin A, and the modification was extended to prepare the special corn Dextrin B. Owing to the low viscosity of Dextrin B, its use made a clay coating with a low adhesive strength at a total solids content of 45%, even though a ratio of 15 parts of dextrin to 100 parts of clay was used. However, as the results in Table XXXIII show, a satisfactory coating was made with 12 parts of dextrin to 100 parts of clay in a coating mixture containing 57.5% of total solids. The coating was found to be weak when 10 parts of dextrin to 100 parts of clay were used in a coating mixture containing 55.7% of total solids.

From the discussion it might be presumed that the adhesive strength of the coating could be increased indefinitely by increasing the ratio of starch product to that of clay, other factors remaining constant. By this procedure one should be able to obtain coated papers that would give very high indices for printing by the Dennison wax test. The difficulty encountered in this procedure with starch products is that, if the concentration of a starch product is increased above a critical limit in respect to the amount of total water present, the coated paper does not develop the desired gloss when it is ironed (calendered) by the finishing rolls. This limit for many starch products, when used in coatings containing about 40% water, is about 20%, based on the weight of clay. This value is for the coating of medium to hard paper stocks; soft, or absorbent paper stocks may require a higher ratio of adhesive.

Improvements in the art of applying machine coatings have permitted the use of coating colors that have greater viscosity and plasticity than the brush coatings discussed previously. For example, it is possible to apply coatings with the newer applicators when the viscosity is of the order of 10,000 to 50,000 centipoises, whereas the viscosities of coatings described in the preceding sections are of the order of 500 to 5000 centipoises. These improvements in machine design have also favored the use of starch products. The special torrefaction Dextrin B, mentioned above, when used in a ration of 20 parts by weight to 100 of clay in a coating color which contained a total of 67% dry solids, developed a viscosity of the order of 50,000 centipoises. This coating was readily applied by special roll transfer applicators on a commercial paper machine and the coated paper gave a Dennison wax test of 8 to 9.

In the newer type of application of the coating, some thixotropy in the coating color is permissible, even desirable (21, 26, 28, 29). As these relatively thick-bodied coatings are metered out and applied to the paper sheet it is desirable for the coating to become fluid enough to form a smooth surface under the pressure exerted by the applicator. A dilatant coating, on the other hand, is obviously not desirable. A thixotropic coating is recognized by a curvature downward in its viscosity curve as obtained from measurements made by methods similar to those previously described. The curvature in the viscosity graph, plotting stress against rate of shear, may be taken as a measure of thixotropy. This value is indicative of the rate of change in the viscosity for a change in the rate of shear, since the tangent to the curve is proportional to the viscosity. For convenience, the viscosity at some specified low rate of shear divided by the viscosity at a specified high rate of shear may be taken as a "thixotropic index" (21).

Many starch products, such as the torrefaction dextrins discussed above, naturally impart the correct order of thixotropy to coating colors at a high solids concentration, and this is a distinct advantage for starch over several adhesives. Casein, for example, naturally imparts dilatancy, the opposite property to thixotropy, to coatings when the solids content is of a high order. Dilatancy causes the coating to become less mobile as shearing stress is applied and in extreme cases the coating solidifies and places a breaking action on the applicator rollers until the stress is released and the coating becomes mobile again. On a high speed machine this cycle may take place automatically, within a fraction of a second, followed by a second, and additional similar cycles. The result is a series of ridges on the surface of the paper instead of a smooth surface.

For an exposition on the colloidal terms, viscosity, plasticity, thixotropy, and dilatancy, particularly as applied to starch-clay coatings, reference is made to the discussion given by Frost (30).

The use of starch modified by enzymes as an adhesive in high density, machine coatings, would appear to present some difficulty owing to the limited amount of water available to make the conversion. In practice, starch is liquefied by amylases by gelatinizing the raw starch in the presence of the enzyme. Ungelatinized starch is attacked only at a very low rate by enzymes and to avoid the uneconomical procedure of heating starch suspensions to pasting temperatures and cooling the mass to some level optimal for enzyme activity, the two operations, gelatinization and conversion, are allowed to proceed together. In the early stages of this method, of course, the starch still has a very high viscosity, that is, requires a high ratio of water to give a fluid mass.

However, in preparing the starch for coatings having a dry solids content of the order of 60 to 65%, then if the clay slip is already fluxed at this ratio of solids to water, the prepared starch sol must be held at this ratio of solids to water also, which is roughly two parts of starch to one of water. Even were the starch already at the level of conversion required for coating work before the addition of any enzyme, it would be a solid mass in the presence of 0.5 parts of water.

By the use of special enzymes that have a high order of thermal stability, introduced commercially in recent years, and by the use of special heating, converting, and homogenizing equipment for the starch, it is possible to convert corn starch in a reasonably uniform manner in the presence of about 3 parts by weight of water. A higher proportion of starch, particularly the less readily dispersible botanical varieties, leads to a non-uniform hydrolysis; some of the colloidal and chemical units are overconverted while others are definitely underconverted. The final result is an uneconomical use of starch. But even using 3 parts of water to one of starch for conversion, and a final ratio of only 15 parts, dry substance, starch to 100 of clay which has been prefluxed in 0.5 parts of water, gives a final dry substance content of only 55%, whereas coatings containing definitely higher solids content may readily be made, using some of the preconverted starch products discussed above.

In order to circumvent the difficulty under discussion, recent patents to Craig (31) propose the addition of raw starch to the clay and then a conversion of the starch by enzymes in the clay mixture. By this procedure it would appear that even at a total solids content as high as 65%, there would be available more than 6 parts of water to hydrate, both physically and chemically, one part of starch, using a normal ratio of starch to clay of 15:100. The error in logic is, of course, that there is not 6 parts of free water to one of starch in the clay mixture; most of the water present has already become asssociated with the clay in order to hydrate it, and scarcely more water is available to hydrate the starch than if both hydrations were performed separately as in the more orthodox procedures. Furthermore, there is the obvious practical objection to this method that the entire mass of clay, water, and starch must be heated to temperatures above the gelatinization range of the starch in order to effect conversion and also, preferably, to about 200-210°F. for some time after conversion in order to inactivate the enzyme. This large mass must then be cooled to a temperature suitable for application of the coating. Hence this procedure involves a considerable, and otherwise unnecessary expenditure for heating and cooling a mass some 5 to 7 times larger than when the starch alone is converted by enzyme. Considering the trend to the use of coatings containing higher solids content and higher operating rates on paper machines and in view of the inherent problem of using enzymes to prepare the starch plus the probable difficulties of uniformly converting the starch under adverse conditions, it would seem preferable to use a starch adhesive already adjusted to the proper viscosity level in starch manufacturing procedures, such as the dextrins mentioned above, which seem to be uniquely suited for high density coating applications.

Landes (32) has pointed out the very great expansion in clay coating paper which has taken place in recent years and that the major share of this expansion has been in machine coating applications.

In some applications, such as offset lithographic printing, a certain measure of water resistance is required in the clay coating. Starch is at a disadvantage compared to casein in the ease with which it reacts to form a water resistant adhesive for the clay in either the brush or machine application procedures. In order to insolubilize these water-soluble colloids, a condensing agent is added which combines to form an insoluble complex. Inasmuch as the colloidal properties of the adhesive change during this transformation, which thereby induces a change in the operational characteristics of the coating color, it is desirable that the latter phases, at least, of the reaction between condensing agent and adhesive take place after the coating has been applied to the paper. The problems involved have been discussed by Felske, *et al.* (33).

Formaldehyde alone has not proven to be very satisfactory with starch in the development of water-resistant coatings. Better results have been obtained by using water-soluble compounds of urea and formaldehyde or melamine and formaldehyde, such as the resins described in patents issued to Osgood (34), Moller (35), Conaway (36), Bock (37), and Howald (38). With these and starch products the reaction is accelerated by acid, particularly below about pH 5, and by higher temperatures. Thus, the coating is formulated usually at a pH value higher than 5.0 in order to retard the reaction within the coating color, and a catalytic agent such as ammonium chloride, oxalate, or phosphate is added just prior to application, which eventually develops an acidity due to combination of the ammonium radical with the formaldehyde in the complex.

In the early stages of the reaction, viscosity of the starch increases greatly and therefore if the reaction occurs prematurely the coating color may become too viscous for application at a normal solids content. Accordingly, both the clay slip and the adhesive are prepared separately as in normal practice and blended. The resin is then mixed into the color at a pH of 5, or above, and lastly the acid-developing catalytic material is added.

Cure of the coating is initiated when the paper is passed to the driers. The reaction is relatively slow ordinarily, so that a heat treatment more intense than is used in normal drying operations is required. However, neither acidity nor temperature can be used without limit to promote the reaction, for beyond critical values, both the base stock and the coating will deteriorate.

Water resistance increases with the amount of added resin and where sufficient water resistance does not develop under particular operating condition, a high ratio of resin to starch may be employed. In most cases, however, a ratio of 15 to 30% resin, based on starch, is sufficient to develop the required wet-rub resistance with the majority of starch adhesives in clay coatings. In general, a higher ratio of starch to clay is required when water resistance is to be developed by the addition of formaldehyde resins than when it is not. Ratios of 25 to 30 parts of starch to 100 parts of clay are commonly reported (33, 39, 40).

It is frequently reported that the use of formaldehyde resins increases the wax test and gloss of the coated sheet in addition to imparting water resistance. It should be pointed out however, that most of the benefits to be derived from the use of condensing agents with starch depend to a large measure on selecting the proper resin, and the proper degree of prepolymerization of that resin, for the particular type of starch modification selected for coating work. In general, the lower the polymerization degree of the starch products used, the higher should be the polymerization degree of the resin.

In common with the action of many condensing agents on starch, the second phase of the reaction with urea-formaldehyde resins gives a product of much lower viscosity than the high viscosity product observed in the initial phases. Kesler and coworkers (41) have outlined a formulating process wherein the starch product is first reacted with the resin, or a part of the resin to be employed, until the stabilized viscosity level has been attained. This reaction mixture is then added to the clay slip with, or without additional resin, depending on operating conditions. In addition to minimizing unduly large changes in the viscosity of the coating color which oftimes results through premature reaction of the starch and resin, this method is reported to provide a coated sheet which is more readily cured than when the adhesive and resin are added separately.

Although other reported means of insolubilizing starch films have been proposed for use in clay coating work (28, 33, 39) little practical information is available as to their merits or as to the extent to which they have been employed. Recently, a monograph discussing in some detail the chemistry and technology of starch in clay coatings has been prepared by a group of qualified workers in both the paper coating and starch manufacturing industries (42).

Sheets (43) has recently presented a critical study of the structural viscosity of coating clay compositions. The results reported should aid an attempt to evaluate the effects of various starches for this use particularly in affording an understanding of variables introduced into the tests by the type of clay and the type and amount of dispersing or fluxing agent used. Several coating clays were studied, and it was established that the differences in the flow properties of the clays are caused by differences in the distribution of particle size in clay species, and in hydrating ability. However, the amount of dispersing agent, such as $Na_4P_2O_7$, used is the most important single variable in altering the flow properties of the clay suspensions studied. When enough dispersing agent has been added to give a minimal viscosity to the clay slip, the next most important variable is the particle size of the clay. Washing the clays with acid tends to make them more non-hydrous. A mild heat treatment irreversibly dehydrated one sample sufficiently to change its flow properties to some extent. Nevertheless, neither acid washing nor heating, singly or in combination, was found to be sufficient to change the flow properties of one clay to those of another.

It was established by Sheets that an intense thixotropic build-up occurs in clay suspensions when certain amounts of dispersing agent are used. The amount of dispersing agent required to give this thixotropy is slightly less than the amount required to give a minimal viscosity to the clay slip. Clays with relatively large particle size may show a marked dilatant behavior over a relatively narrow range of clay concentration when enough dispersing agent has been added to these clay slips to secure a minimal viscosity.

For fractions of clay from the same species, the general conclusion may be drawn that clays of smaller particle size give a thinner suspension for a given solids content than clays having a large particle size. This is particularly true for concentrated clay suspensions such as are used in machine coatings. This result should be considered, when it is desired to use the less expensive modifications of starch as the adhesive in machine coatings.

Hauser (44) has discussed in some detail the colloid chemistry of clays.

Sutermeister (45) has recently reviewed the literature on the sizing of paper with starch products. Casey (46) has traced past trends in the use of starch in the paper industry and has outlined possible future developments in this field.

BIBLIOGRAPHY

1. Lee, H. N., *Tech. Assoc. Papers* (TAPPI), **21**, 319 (1938).
2. Casey, J. P., *Paper Trade J.,* **114**, 107 (1942).
3. Rowland, B. W., and Bauer, J. V., U. S. Patent 2,113,034 (1938).
4. Rowland, B. W., U. S. Patent 2,207,555 (1938).
5. Kesler, C. C., and Black, W. C., *Paper Trade J.* (TAPPI), **114**, 247 (1942).
6. Heald, A. M., *Paper Trade J.,* **113**, 39 (1941).
7. Cobb, R. M., Lowe, D. V., Phol, E., and Weiss, W., *Tech. Assoc. Papers* (TAPPI) **20**, 299 (1937).
8. Kerr, R. W., U. S. Patent 2,108,862 (1938).
9. Casey, J. P. *Paper Ind. and Paper World,* **27**, 1277 (Jan. 1945).
10. Kerr, R. W., U. S. Patent 2,468,207 (1949).
11. Kerr, R. W., *Paper Trade J.* (TAPPI), **115**, 30 (1942).
12. Tanret, C., *Compt. rend.,* **158**, 1353 (1914).
13. Pacsu, E., and Mullen, J. W., *J. Am. Chem. Soc.,* **63**, 1168 (1941).
14. Samec, M., *Ber.,* **73A**, 88 (1940).
15. Kerr, R. W., and Schink, N. F., *Paper Trade J.* (TAPPI), **120**, 86 (1945); see also Chemistry and Industry of Starch, Academic Press, New York, 350, 1st ed. (1944).
16. Kerr, R. W., and Severson, G. M., *J. Am. Chem. Soc.,* **65**, 193 (1943).
17. Fowler, G. B., Indian Patent 29,375 (1944); see also, U. S. Patent 2,385,438 (1945).
18. Brimhall, B., Dissertation, Iowa State College, Ames (1943).
19. Kerr, R. W., Meisel, H., and Schink, N., *Ind. Eng. Chem.,* **34**, 1232 (1942).
20. Gillan, E. P., *Paper Trade J.* (TAPPI), **115**, 109 (1942).
21. Kerr, R. W., and Schink, N. F., *Paper Trade J.* (TAPPI), **120**, 77 (1945).
22. Diehm, R. A., *Tech. Assoc. Papers* (TAPPI), **20**, 279 (1937).
23. Saxl, I. J., *Tech Assoc. Papers* (TAPPI), **20**, 340 (1937).
24. *Paper Trade J.* (TAPPI), **115**, 220 (1942).
25. Casey, J. P., *Paper Ind. and Paper World,* **26**, 157 (May, 1944).
26. K-C-M Co., British Patent 545,124 (1942).
27. Massey, P. J., *Paper Trade J.,* **120**, 39 (1945).
28. Haywood, G., in Starch for Paper Coating, edited by R. T. Bingham, TAPPI, New York, 47 (1947).
29. Kerr, R. W., Chemistry and Industry of Starch, Academic Press, New York, 364 (1944).
30. Frost, F. H., in Starch for Paper Coating, edited by R. T. Bingham, TAPPI, New York, 105 (1947).
31. Craig, W. L., U. S. Patents 2,360,828 (1944), 2,388,526 (1945), and 2,394,233 (1946)
32. Landes, C. G., *Paper Trade J.* (TAPPI), **123**, 39 (1946).
33. Felske, F. G., Gardner, H. F., Getty, J. E., and Kesler, C. C., in Starch for Paper Coating, edited by R. T. Bingham, TAPPI, New York, 73 (1947).
34. Osgood, G. H., U. S. Patent 2,141,213 (1938).
35. Moller, F. A., U. S. Patent 2,242,635 (1941).
36. Conaway, R. F., U. S. Patent 2,254,001 (1941).
37. Bock, L., U. S. Patent 2,301,509 (1942).
38. Howald, A. M., U. S. Patents 2,334,096-7 (1943).
39. Hewett, P. S., Carter, R. E., and Church, R. J., *By Gum!* (Reichhold Chemicals Inc.) **17**, 7 (1947); *Paper Trade J.,* **124**, 45 (1947).
40. Landes, C. G., U. S. Patent 2,399,489 (1946).

41. Kesler, C. C., Killinger, J. E., and Hjermstad, E. T., *Paper Trade J.*, **122**, 39 (1946). See also, U. S. Patent 2,450,377 (1948).
42. Bingham, R. T., Starch for Paper Coating, TAPPI, New York (1947).
43. Sheets, G. H., *Tech. Assoc. Papers* (TAPPI), **25**, 528 (1942).
44. Hauser, E. A., *Chem. Revs.*, **37**, 287 (1945).
45. Sutermeister, E., *Paper Ind. and Paper World*, **29**, 1761 (1948).
46. Casey, J. P., *Paper Ind. and Paper World*, May 28, 268 (1946).

CHAPTER XIX

USES OF STARCH AND STARCH PRODUCTS IN THE FERMENTATION INDUSTRIES

BY LEO M. CHRISTENSEN

1. **Introduction.** The fermentation industries constitute a branch of chemical manufacture in which starches and sugars are converted by use of the enzymes of yeasts, molds and bacteria into a number of useful products. The best known of these chemical products, and the one produced in largest volume is ethyl alcohol, but other fermentation chemicals, including *n*-butanol, acetone, lactic acid, citric acid, gluconic acid, itaconic acid, glycerol and 2,3-butylene glycol, are of large present or potential importance. The current large production of antibiotics, of which penicillin and streptomycin are best known, is an outstanding development in the fermentation industries. Research into the production of other chemical products by fermentative processes is active in many laboratories. The index to these fermentation chemicals presented by Fulmer and Werkman (1) shows the wide range of products that may be prepared by these means, and many have been discovered since publication of this report in 1930.

Chemical manufacture is characterized by a high degree of flexibility as to raw materials used and does not hesitate to take advantage of changes in prices. Blackstrap molasses, a byproduct from the manufacture of cane sugar, was the lowest cost raw material for the fermentation industries during most of the 25 years preceding World War II. Its production is limited by the amount of sugar produced, the yield is about 50 gallons per long ton of raw sugar, and since world sugar consumption has apparently reached a rather stable level, there is no indication of large increase in supplies. But in 1935 a new kind of molasses came into the markets. Cuban cane production was in excess of market requirements and the whole juice from this surplus was inverted by heating at acid reaction, neutralized, and evaporated to 70% sugar content. Blackstrap is normally sold on a 55% total sugars basis. Imports of blackstrap and hightest molasses in the years preceding World War II, as reported by Gabriel (2), are shown in Table XXXIV.

If cane acreage and yield remain constant, blackstrap production increases and hightest decreases as sugar consumption expands. It seems probable that cane acreage may increase faster than markets expand, continuing the prewar trend. Thus, increasing amounts of hightest may be expected to become available. This may be fully offset, however, by the steadily increasing use of molasses in the countries of origin for the manufacture of alcohol for motor fuel. Cuba, Brazil and other molasses-exporting nations are now using motor

TABLE XXXIV

Imports of Blackstrap and High Test Molasses in Millions of Gallons

Year	Blackstrap	Hightest	Total
1935	123	59	182
1936	127	116	243
1937	150	208	358
1938	145	89	234
1939	150	100	250
1940	125	178	303
1941	101	339	440

fuels containing up to 75% ethyl alcohol made from molasses. The sad state of most national currencies prevents the establishment of a clear cut picture. It is highly significant, however, that molasses prices delivered at United States ports continue at high levels, three to four times prewar, and supplies are short. Gabriel (2) is convinced that blackstrap or hightest molasses will eventually become available at substantially prewar volume and price.

Ethanol has been made from byproduct ethylene, and isopropanol and acetone have been produced from propylene by the petroleum industry for a number of years. Burger (2) has pointed out that increasing demands for ethylene, ethane and propane in the manufacture of other commodities can be expected to have an important effect upon the manufacture of these synthetics in the near future. Some of these new products will tend to replace ethyl alcohol, as for example, the oxidation of hydrocarbon gases to acetaldehyde.

There is currently a great deal of interest in the alcohols that will be produced from operation of Fischer-Tropsch plants, two of which are now being built. This process can produce from carbon monoxide and hydrogen a wide range of products, depending upon the catalyst, the CO-H_2 ratio, temperature and pressure. Schroeder (2) has described the general situation and has presented cost data indicating that by this means very low cost alcohols may soon become available. It is probable that the present plants will produce gasoline and Diesel fuels as primary products and will yield alcohols, aldehydes and other oxygenated compounds as byproducts. The major handicap in this development is the very large plant investment required.

The manufacture of alcohols and other chemicals from starch reached a high level during World War II. Boruff and Van Lanen have summarized the production data (3). The total ethanol production reached 606 million gallons in the United States in 1944, as compared with 125 million gallons in 1941. Seventy per cent of the synthetic rubber produced in the war period was made from ethanol, and 70% of this alcohol was made from grain starches, according to data released by the Rubber Reserve Company (4). The grain consumption for ethanol, as reported by Jacobs (5) is shown in Table XXXV. Since the war there has been a large decline in the manufacture of grain alcohol, as a result of very high grain prices caused by government purchases for foreign distribution, curtailment of grain use by government order, and reduction in the amount of alcohol used for synthetic rubber. The recent very high alcohol prices, industrial alcohol being quoted at about $1.00 per gallon as compared with the normal prewar price of $0.22, has prevented the use of this and other fermentation chemicals in new products and curtailed the use in old industries.

TABLE XXXV

Grain Consumed for Industrial Alcohol During the War Years

	Consumption in millions of 56 pound bushels			
Year	Corn	Wheat	Sorghums	Total
1942	40.0	1.5	0.5	42.0
1943	50.0	45.0	0.3	95.3
1944	4.0	113.0	10.8	127.8
1945	29.0	83.0	49.0	161.0
Total	123.0	242.5	60.6	426.1
Per cent	28.7	57.0	14.3	100.0

Ethanol prices probably will decline from recent high levels, perhaps not to the levels of the prewar period, but surely to something like one-third of recent values. Can the manufacture of these chemicals from starch meet the competition of manufacture from molasses and by synthetic processes? Obviously, this depends upon many factors, and particularly upon the efficiency of the manufacturing processes.

2. **The Agricultural Situation.** In the prewar period it was the commonly accepted view that American agriculture had a productive capacity 12% in excess of domestic and foreign markets. Agricultural productivity expanded during the war, through technological advances, by 36%, according to several government estimates (6). Recently there has been a large movement of grains abroad, but it is well known that this has been primarily due to the instability of European currencies and our efforts to develop stable governments, and is not the result of real food shortages. Thus it is evident that this artificial demand will end in the near future. It seems entirely probable that foreign markets will assume approximately the prewar proportions. The domestic market probably will be only a little more than prewar, in which event the eventual postwar surplus may well reach

48% of the total grain crop. This is a common opinion among agricultural interests.

But this is not a true surplus of grains. The analyses by Hale (7), Christensen, *et al.* (8), Shepherd, *et al.* (9), Willkie and Kolachov (10) and Filley, *et al.* (11) have shown that all of the components of this so-called surplus are needed in the national economy and have had to be imported to meet requirements, except the starch. The problem, then, is one of finding uses for this starch. The use in fermentation processes is, of course, of great interest in this connection and is the subject of several recent analyses. Those by Groggins (2), Christensen (11), Jacobs (5), and Hilbert (12) are good indications of the current thinking in agricultural groups.

3. **Prospects for New Markets.** The most interesting of the prospects for new markets for fermentation chemicals centers around ethanol. From the standpoint of potential magnitude, the use of ethanol as a supplemental fuel for internal combustion engines is of outstanding interest, and in particular its use in a dual fuel system seems especially attractive. The technical and economic aspects of this system have been discussed by Colwell, *et al.* (13) and by Hilbert (12).

In this system the ethanol is carried in a separate tank and is admitted to the intake manifold by means of an automatically controlled metering system that adds large amounts when conditions are favorable to detonation (open throttle and relatively low piston speed in most engines) and only small amounts under other conditions. Depending upon the price of ethanol, it may be used in a 50% aqueous solution or higher concentration. It should be pointed out that methanol and isopropanol can also be used in this manner and that the three alcohols have substantially the same value for this purpose. This system provides a substantial improvement in antiknock value and cleaner and more efficient combustion, resulting in better mileage and improved performance. Small scale commercial distribution is now operating and there was an extensive use of this system in aircraft during the late war.

It is particularly interesting to note how well this system is adapted to use with very high compression ratio engines, such as the one developed by Kettering and his associates and described in recent reports. Theory indicates, and apparently tests confirm, that by use of compression ratios of 10 to 1 and higher, fuel consumption by automotive equipment might be reduced by as much as 50% per unit of operation, as compared with present equipment. This would be a highly desirable attainment in view of the current domestic petroleum shortage and rapidly expanding consumption.

It is hazardous to forecast prices in the present unstable economy, but if gasoline prices remain as at present, it seems probable that alcohol may be able to command a price of $0.40 to $0.50 per gallon in this market. If all the automotive equipment in use in continental United States used the system, the consumption of ethanol would reach 1,000 million gallons per year.

Brooks (14) has reported the very thorough research by the National Bureau of Standards on the fuel value of ethanol, *n*-butanol, acetone and various mixtures of these compounds. Power and economy tests were made at several compression pressures, mixture strengths, and spark advances with each fuel, and with two gasolines, in a single cylinder engine. When used under optimum conditions for power output, these alcohol and acetone fuels gave considerably higher power output and higher thermal efficiencies than were obtained with gasoline. This is, of course, the reason alcohol fuels are so commonly used in racing engines.

Expanding applications of ethyl acetate, ethyl cellulose and other plastics using ethanol, open new markets in this field, and there are trade reports of other new plastics that are based upon the use of ethanol, acetone or *n*-butanol.

Lactic acid is another fermentation chemical of interest for plastics but no large developments seem imminent, according to Wetzstein (15). Any expansion in the utilization of lactic acid would seem to be dependent upon the development of methods which result in lower cost of production. Recovery from relatively dilute solutions is not yet on a good economic basis.

Itaconic acid, a new commercial fermentation product, is an attractive chemical intermediate for plastics and other commodities.

Research on production and use of 2,3-butylene glycol has been very active during recent years. This chemical is of interest as a source of butadiene, for chemical synthesis and the levo form, as produced by *Aerobacillus polymyxa* from starch, is interesting as a permanent antifreeze.

Production of antibiotics by various microorganisms is rapidly expanding, and new products are being developed by intense research in this field.

Recent research has been active on the production and use of enzymes derived from bacteria and molds. The old observation that the addition of fungal amylase preparations to poultry rations has a beneficial effect (16) is receiving careful study in industrial laboratories.

Several plants recover riboflavin concentrates from bacterial and yeast growth, and research in this field is in progress in many industrial laboratories. In general these are byproducts at present, but this may not always be the case. Langford (17) reported that by the use of *Ashbya gossypii* in a suitable substrate riboflavin concentrations of 700 micrograms per milliliter could be obtained, and that the solids contained can be recovered by evaporation and drying without loss of riboflavin.

4. Ethanol. The fact that the manufacture of ethanol from starchy

substrates is probably the oldest organic chemical industry, and has always had a prominent place in the industry, has led to the assumption that it must be operating at a high degree of efficiency. Beresford and Christensen (18), Wilkie and Kolachov (10), and Christensen (19) have shown that this is far from true. Calculation shows how poor the conversion efficiency is. Thus, by orthodox procedures, yields commonly obtained are, 15.7 lbs. of ethanol (4.75 proof gallons or 2.50 wine gallons of 190 proof ethanol) and 14.8 lbs. of dry residue (16.5 lbs. of 10% moisture) per bushel (56 lbs.) of grain of 12% moisture content (49.3 pounds of dry substance). This is 31.8 pounds of ethanol and 30.0 pounds of dry residue per 100 pounds of moisture-free grain. One mole of $C_6H_{10}O_5$ yields two moles of ethanol and two of CO_2, and 1.761 pounds of starch are required to yield 1.0 pound of ethanol and 0.95 pound of CO_2. All the dry substance not fermented should be recovered as residue. But,

$$(31.8 \times 1.761) + 30.0 = 86.0$$

or 14 pounds less than expected. That is, by this accounting, the process is only 80% efficient, assuming 70% starch in the grain.

What happened to the 14 pounds? Fragmentary and circumstantial evidence shows that the loss is in the carbohydarte fraction and that through some little-known kind of retrogradation a part of the starch is converted during cooling following cooking to a form that yields carbon dioxide and water during fermentation, distillation and drying of the residues. It is indicated that dicarboxylic acids are intermediates, and are formed early in the fermentation. It has been noted that where such retrogradation has occurred, there is a considerable evolution of CO_2 during fermentation that is not accompanied by alcohol formation, and a larger carbohydrate disappearance than theoretically required for the amount of alcohol produced. Titratable acidity is higher where there has been retrogradation but the H-ion concentration is little affected. The x-ray diffraction pattern and viscosity data also are indicative of the change in the starch and show that it starts in the cooling process.

Four ways have been found to avoid or minimize this starch loss. Christensen (20) described a continuous cooking process and apparatus using instantaneous mash cooling by dilution with cold amylase slurry or solution of proper concentration and temperature. This process has had large scale commercial application. During this operation, a thorough study was made to determine the influence of the mash temperature at the entrance to the mash-water-amylase mixer. Laboratory research had indicated that the critical temperature, below which retrogradation occurs and above which it is very small, is around 80°C. (176°F.). Underkofler (21) found in this study that ethanol yields were 4.6% higher when the entrance mash temperature was 80°C. (176°F.) or higher than it was when the temperature was below this level, with rather a sharp break between 80 and 76°C. Because of lack of adequate facilities, the yield of residual solids could not be measured, but laboratory measurements indicated a substantial gain due to minimizing the starch retrogradation. By this method, the ethanol yield was raised to 84% of that theoretically possible as calculated above.

A modification of this process was developed by Christensen and Taylor (22). In this process one-fourth of the total amylase is added to the mash in a solution or slurry to yield a mixture temperature of 78°C. (172°F.) minimum, then the mash is cooled to 55-60°C. (131-140°F.) by any means, and the balance of the amylase is added. This process was applied to potatoes, resulting in an increase in alcohol production of 32% per unit of potatoes processed, as compared with orthodox cooling and saccharification. This semi-commercial operation is described by Beresford and Christensen (18).

A third process described by Christensen (23) involves removal of certain grain germ components, cooking at an acid reaction, and for a time at a temperature so that approximately 12% of the starch is converted to fermentable sugars, then completing the conversion by use of fungal or other suitable amylases. Materials normally present in the germ yield toxins during cooking, but are removed by expelling or by solvent extracting the oil, and are found concentrated in the foots. There is a definite optimum acid concentration at each combination of time and temperature, the optimum being about 0.08 normal, or at a mash reaction of pH 1.8 to 2.2, for temperatures of 120-150°C. (248-302°F.) and cooking times of around 20-30 minutes. With lower acid concentrations retrogradation occurs, while at higher concentrations there is a tendency for the dextrose to repolymerize, or revert, yielding unfermentable polymers. Properly applied in the laboratory, this process has regularly yielded 40.0 pounds of ethanol (12.1 proof gallons) per 100 pounds of dry corn, or about 25% more than orthodox procedures. This is very nearly the theoretically possible yield.

The fourth method is described by Severson, *et al.* (24). It uses a precooking treatment of the grain slurry with thermodural bacterial amylases made by the methods of Beckord, *et al.* (25) or Beckord, *et al.* (26). The bacterial amylase is added to the grain slurry at approximately 55°C. (131°F.) at a reaction of pH 5.5 to 6.0 and then heated at such a rate that in 20 minutes the temperature is 85°C. (185°F.). Then the mash is cooked and saccharified, preferably continuously and with cooling by dilution with fungal amylase. This process has yielded 37.0 pounds of ethanol per 100 pounds of dry corn, or about 16% more than orthodox procedures. This process has had successful pilot plant application.

A major cost in making ethanol from starchy substrates arises from the cost of the required amylase, ordinarily barley malt. Several much cheaper and more effective preparations, adapted to manufacture at the alcohol plant, have had thorough commercial trial. The Amylo process, described in detail by Grove (27) and by Owen (28), obtained saccharification by growth of certain species of *Mucor* or *Rhizopus* in the cooked grain mash at 35° (95° F.), with a high degree of aeration. An Anamite yeast was commonly used because its optimum temperature is around this level. Ethanol yields of 33 to 36 pounds per 100 pounds of dry grain were commonly reported. The major disadvantages were the high mash viscosity in the coolers, the serious danger of contamination during the long incubation, and the difficulties of controlling the mold growth.

Conversion with acid has been quite thoroughly investigated in many plants and laboratories. Severson (29) has presented the results of an especially adequate study of the influence of acid concentration, time and temperature. The data are conclusive in showing that acid saccharification alone is inadequate. If acid concentration, time and temperature are so adjusted that there is the required minimum of residual dextrins, there is a reversion of dextrose to unfermentable carbohydrates, unless uneconomically dilute mashes are used. Under any conditions of practical interest, ethanol yields do not exceed 75% of theoretical. As pointed out above, cooking at acid reaction properly applied can be used to advantage when final conversion is effected by use of enzymes.

Underkofler *et al.* (30) have described the commercial manufacture of a fungal amylase (mold bran) by growing *Aspergillus oryzae* or other molds on wheat bran and similar fibrous substrates. In this process, the bran is mixed with dilute acid to yield a reaction of approximately pH 4.2 and a moisture content, after cooking 30 to 60 minutes at 100° C. (212° F.), of 50-55%. The bran is cooled to 35° C. (95° F.), inoculated with a heavily sporulated culture at a rate of around 0.1% by weight, and incubated in 6- to 9-inch layers with forced air cooling or in 2-inch layers with cooling by diffusion and radiation. Incubation of 24 to 36 hours is adequate. The product may be used directly or may be dried and stored for future use. Underkofler *et al.* (31) have described the successful large commercial use of this mold bran. Used to replace malt in yeast culture and in fermenter mash, it has provided a yield increase of around 5% as compared with malt.

Fungal amylases made by use of liquid substrates have more recently become of interest. Thin stillage has been the preferred substrate. Erb and

Hildebrandt (32) used *Rhizopus boulard* or *Rhizopus delemar* and added a little ground grain, some nutrient salts and powdered aluminum to the stillage, the latter serving to remove or neutralize some unknown toxin normally present. Aeration was required during incubation. This preparation was used at 6 to 12% of mash volume in commercial scale operations to replace 80% of the normal malt requirement, with no substantial change in ethanol yield.

Adams *et al.* (33) and Langford (17) have described the preparation of a fungal amylase by growing a selected strain of *Aspergillus oryzae* (NRRL 337) in thin stillage plus 1% corn meal plus 0.5% $CaCO_3$. This substrate is cooked, cooled, and inoculated with the culture at a ratio of 2% by volume, and incubated with aeration at one volume of air per minute per four volumes of mash and at a temperature of 35°C. (95°F.) for 60 hours. One volume of the product is adequate for conversion of nine volumes of mash of normal commercial concentration. Ethanol yields reported were approximately 5% above those obtained using malt. The process has had adequate pilot plant testing and is reported scheduled for early commercial use.

Back *et al.* (34) have shown that amylopectin is converted to fermentable sugars less completely and more slowly than is amylose when barley malt is the source of amylases. A slow mash cooling process was used. But Underkofler and Wimmer (35) found that waxy sorghums yielded more alcohol than did non-waxy types when conversion was effected with fungal amylases following the instantaneous cooling procedure. Whether the difference indicates that fungal amylases are superior in their ability to hydrolyze amylopectin or indicates that amylopectins are the more susceptible to retrogradation is not clear. The fact that slow mash cooling results in the loss of approximately 20% of grain starches (non-waxy types) and up to 35% of potato starch is of some significance in this connection.

During the war operations, a plant at Detroit started the production of alcohol from wheat starch following recovery of the gluten content. So far as is known, this gave the first commercial evidence of the action of the amylase-inhibitor described by Kneen and associates (36). Wheat was used in other plants and no evidence of the inhibitor activity was noted, presumably because of the inactivation by cooking in the presence of bran and larger amount of protein, as would be predicted from Miller and Kneen's (37) data. Christensen and Taylor (38) found that mold bran used alone or used to supplement malt eliminated the effect of the inhibitor and gave a much larger improvement in yield than has been noted with corn or other grains not containing amylase inhibitors.

It should be noted that the improvements in mashing and saccharification procedures have not only provided a marked improvement in ethanol yields but have also permitted large reductions in processing costs, through larger factory throughput per unit of investment and per man-hour of labor and reduction in steam, water and power consumption resulting from the use of higher mash concentrations. As noted above, there are now at least two amylase preparations in commercial use that cost considerably less than barley malt. Improvements have also been made, and more are in prospect, in byproduct recovery. As a result it is indicated that the cost of making ethanol from starchy substrates will be very greatly reduced in the near future.

5. *N*-Butanol and Acetone. The manufacture of *n*-butanol and acetone by the fermentation of starchy substrates with *Clostridium acetobutylicum* was started in England before World War I, with the objective of producing synthetic rubber. The operation has been described by Perkin (39) and involved the use of Fernbach's fermentation process, Perkin's methods for producing butadiene and isoprene and Matthews' sodium polymerization scheme. The raw material was cull white potatoes. In 1914 the British Admiralty, desperately in need of acetone for cordite, took over the project and put Weizmann in charge of a program which led to transfer of operations to Canada and later to the United States where corn was available. This development has been described in detail by Gill (40).

With the end of the war, demand for acetone declined to such an extent that the operations came to an end. But the development of nitrocellulose lacquers for automobile finishes soon after that led to a very large demand for *n*-butanol, and the stocks stored during the war were soon depleted. An American corporation purchased the plant at Terre Haute, Indiana, acquired the patents issued to Weizmann and later built a larger plant at Peoria, Illinois, and plants in England and in France. The rapid expansion of this industry has been described by Killefer (41), Gabriel (42), Gabriel and Crawford (43), and Wynkoop (44). With the expiration of the basic patents, this industry now includes at least two other firms in the United States and one in the West Indies. While both *n*-butanol and acetone are produced synthetically, the fermentation process has been able to hold its place in the field. This has been possible through the development of byproducts including methanol from the CO_2 and H_2 produced during fermentation and vitamin concentrates from the stillage.

The yield of "total solvents" from corn varies in practice from 26 to 28 pounds per 100 pounds of dry substance processed, or 36 to 39 pounds per 100 pounds of starch. *Clostridium acetobutylicum* produces amylase in adequate amounts for its needs and the mash used contains from 6 to 8% corn or 4 to 6% starch. The final "solvents" concentration is 2.0 to 2.5% by weight, and consists of 60 to 62% *n*-butanol, 29 to 31% acetone and 8 to 11% ethanol.

Little is known about the chemical mechanism but acetic and butyric acids are known to be included in the intermediates, and formic acid has been isolated from the fermenting mash. The overall process may be represented by the following reactions:

$$C_6H_{10}O_5 + H_2O \rightarrow C_4H_9OH + 2\ CO_2 + 3\ H_2$$
$$C_6H_{10}O_5 + 2H_2O \rightarrow (CH_3)_2CO + 3\ CO_2 + 4\ H_2$$
$$C_6H_{10}O_5 + H_2O \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Severson and Christensen (45) found that *n*-butyraldehyde added to the fermentation was quantitatively reduced to *n*-butanol. Acetaldehyde yielded ethanol and acetone in equimolar amounts. Acetic acid was converted to acetone in a yield of one mole of acetone from two of the acid, according to Severson (46), while butyric acid yielded *n*-butanol, acetone and ethanol in a ratio quite like that obtained from starch. Christensen (47) found that increasing the hydrogen pressure, with removal of CO_2, increased the proportion of *n*-butanol, and Johnson *et al.* (48) found that the use of a reduced sugar, mannitol, resulted in a larger proportion of *n*-butanol.

Some strains of *Cl. acetobutylicum* are susceptible to a condition known as "sluggishness," characterized by a normal production of acids in the early stages and a failure of conversion of these to neutral products in the later stages of fermentation. A poor solvent yield results. Legg (49) described the causitive factor as a filterable, reproducing, thermolabile virus resembling a bacteriophage. Starr (50) further described the phenomenon, confirming Legg's findings, and found also that the filtrate did not cause lysis or otherwise affect the morphology. Its effect seemed confined to the metabolic processes.

Prior to 1935 all attempts to produce *n*-butanol and acetone from sugar solutions were unsuccessful from a commercial viewpoint. Then it was found that other related bacterial strains could utilize sugars and at least 12 patents on the use of such bacteria have been issued, of which the five cited (51-55) are the most recent and perhaps the most important. Between 1938 and 1942 blackstrap molasses became the major raw material in this industry but during the war years grains, especially corn, regained this market. At present the picture is confused because of instability of national currencies, resulting in erratic raw material prices, and other factors.

Cl. acetobutylicum is able to ferment pentoses and there have been a number of attempts to use hydrolyzates from corn cobs and other agricultural wastes (56-58). The recent accomplishments in this field by Dunning and

Lathrop (59) may lead to commercial applications. Research to date indicates that these hydrolyzates can best be used in admixture with grains.

6. **Glycerol.** The production of glycerol by yeast as a minor byproduct in the normal conversion of sugars to alcohol was noted by Pasteur but its recovery in commercial operations has not been feasible because of the small yield, usually less than 3.5% of the sugar fermented, and low concentration, of the order of 0.5% in the mash. Neuberg and coworkers (60, 61) described three general courses of ethyl alcohol fermentation by yeast as follows:

1 Normal, in acid media

$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2$

2. In presence of sulfites

$C_6H_{12}O_6 \rightarrow CH_3CHO + CO_2 + C_3H_5(OH)_3$

3. In alkaline media

$2C_6H_{12}O_6 + H_2O \rightarrow C_2H_5OH + CH_3COOH + 2\ CO_2 + 2\ C_3H_5(OH)_3$

On the foundation of this basic research, Connstein and Ludecke (62) developed the process that was used in Germany during World War I to supply badly needed glycerol. Beet sugar was the raw material and large amounts of sulfite were employed. Cocking and Lilly (63) developed a process in England using a mixture of sulfite and bisulfite. An alkaline fermentation process was developed in the United States by Eoff (64). Detailed descriptions of these processes are given by Eoff *et al.* (65), Ling (66), May and Herrick (67) and Nord (68).

In actual practice, using either the sulfite or the alkali process, the yield of glycerol is considerably less than predicted by Neuberg's equations, ranging from 10 to 15 pounds per 100 pounds of sugar with the alkali method to as much as 35 pounds by the sulfite process. The sulfite process requires an amount of sodium sulfite equal to or greater than the amount of sugar processed, and is considerably more expensive in respect to factory costs. In addition, both proceses yield a low quality glycerol and at uneconomically low concentrations.

The large increase in demand for glycerol for glyptal and similar protective coating resins and for other chemical operations has resulted in the impossibility of satisfying requirements by the production in soap manufacture. Research on improved methods for making glycerol by fermentation has developed more economical processes. Underkofler *et al.* have developed a process using calcium or magnesium sulfites (69) and another using ammonium sulfite (70). The sparingly soluble alkaline earth sulfites are especially well-suited to this process because their use greatly simplifies the recovery of the products. In both processes the sulfite ion content is maintained by adding SO_2. Yields are reported on the basis of 100 pounds of dextrose as 25 pounds glycerol, 7 proof gallons of ethyl alcohol and 10 pounds of acetaldehyde.

In the manufacture of glycerol from starchy substrates, it is necessary to convert the starch to dextrose rather than maltose, and thus fungal amylases are especially useful. Preferably the starch should be separated from other grain components prior to saccharification, but it is also possible to saccharify and then remove the suspended solids by centrifugation and filtration.

7. **Lactic Acid.** Although lactic acid was apparently produced commercially in the United States as early as 1881 by Avery at Littleton, Massachusetts, there was no important manufacture in this country until about 1940. Production increased to nearly nine million pounds in 1944, but has now declined to about half this level. In the past, most of the supplies have come from Europe. The dark grades are used in the leather industry while better grades are employed in food products. Research has shown other opportunities for uses of the acid, metal salts, esters and ethers, as described by Smith and Claborn (71), and current interest indicates possible large scale use in acrylate plastics (72-7).

Starch is the source of the major part of the lactic acid produced, although small amounts are also made from whey and from molasses. Most of the production is obtained in connection with corn products manufacture and a starch-rich fraction is employed as the raw material. The manufacturing process has been described by Peckham (78). The starch is hydrolyzed by use of a dilute mineral acid at elevated temperature and a fungal amylase may be employed to complete the conversion. Malt sprouts or steep water are used as accessory nutrients. The sugar concentration is normally 12 to 18%. The fermentation is carried out at 50°C. (122°F.) using *Lactobacillus delbrückii* or *L. bugaricus*. The acid is neutralized by periodic addition of calcium or zinc carbonate or hydroxide.

It has long been known that mixed cultures of thermophilic bacteria, easily prepared by inoculating cellulose-mineral salts media with manure, rotten wood or soil, are able to ferment starchy substrates directly to give good yields of lactic acid. So far as can be learned, no commerical application has been made.

The dark grades of the acid are simply prepared by adding sulfuric acid exactly equivalent to the calcium, filtering, clarifying, decolorizing and evaporating at low temperature to the desired concentration. Preparation of the purer grades is a considerably more difficult operation. The zinc lactate may be crystallized from the solution, purified by repeated recrystallization, the lactic acid freed by precipitation of zinc sulfide, and the acid purified by treatment with charcoal and filtration. Another process involves crystallization of the calcium lactate, which is washed and then dissolved in hot water. Calcium is removed by addition of the exact equivalent of sulfuric acid and the lactic acid solution is decolorized with charcoal and concentrated in a vacuum evaporator. More recently the recovery has been effected by esterifying the lactic acid with methanol and carefully fractionating or by extractting with a suitable immiscible solvent, such as isopropyl ether (79) or chlorinated hydrocarbons (80).

8. Butylene Glycol. It has long been known that many bacterial species produce 2,3-butylene glycol or its oxidation products, acetylmethyl carbinol and diacetyl, from starches and sugars. Breden and Fulmer (21) and Fulmer, *et al.* (82) found that by proper control of the reaction and of the sugar concentration yields of glycol as high as 50% of the sucrose processed could be obtained by the use of *Aerobacter aerogenes*. Kluyver and Sheffer (83) obtained a patent on a process for commercial production of the glycol. Christensen (94) found that sugar concentrations below about 6% favor lactic acid production but that between that level and as high as 27% sugar the glycol yield approached 50% by weight of the sucrose consumed.

Hill and Isaacs (85) described the pyrolysis of the glycol diacetate to yield butadiene, and Elder (86) reported yields of 88% of the theoretical in pilot plant applications of this procedure.

The war-induced rubber shortage greatly stimulated research in this field, especially in the United States and in Canada. Coghill and assosciates (87, 88) reported fermentations of hydrolyzates from grains with *Aerobacter aerogenes* yielding 40 pounds of the glycol per 100 pounds of original starch, and found that the product was primarily of the meso form. They found also that trace elements naturally present in the hydrolyzate, especially manganese, cobalt and molybdenum, acted to depress the glycol yield, and recommended their removal by ion exchange methods.

Ward *et al.* (89) found that *Aerobacillus polymyxa* produced the levo form of the glycol, with ethanol as a side product, by direct fermentation of starchy substrates. The glycol-ethanol ratio is normally between 1.5 to 2.0 to 1.0. While the mash concentration must be low as compared with the *Aerobacter aerogenes* fermentation of sugars, with the optimum around 12% of starch, the elimination of the need for saccharification led to concentration of research on this process, especially in Canada. The twelve references cited (90-101) include most of the reports of the detailed investigations. In this process the grain mash is cooked by normal processes, inoculated with an active culture and fermented without aeration.

Neish, *et al.* (102) found that Ford's strain of *Bacillus subtilis* (NCTC 2586 or ATCC 2586) produced from 100 moles of $C_6H_{12}O_6$, 57 moles of glycol, 40 of glycerol, 20 of lactic acid, 13 of ethanol and 5 of formic acid. Adams (103) reported the production of the glycol (levo and meso mixture) and acetic and lactic acids by the fermentation of glucose or xylose by *Aeromonas hydrophila.*

Research on the recovery of the glycol has also been reported in detail. Strohmaier and Lovell (104) described optimum conditions for clarification of the mash before and after fermentation. Walmsley and Davis (105) patented a process combining evaporation and steam distillation. Cornwell (106) was issued a patent based upon separating the glycol by dialysis. Senkus (107) removed the glycol as the formal, from which he obtained the glycol by treatment with methanol. The process that had the most thorough pilot plant study involves removal of ethanol by distillation, clarification by centrifugation and filtration, evaporation to approximately 20% glycol concentration and steam distillation of the glycol. Recovery of the glycol by liquid-liquid extraction from the 20% solution has attractive possibilities that have not yet been fully explored (113).

Kolfenbach (108) found that direct dehydration of the glycol by catalytic processes gave low butadiene yields. The pyrolysis of the diacetate was very thoroughly investigated in two pilot plants and the findings are described in detail in a series of eight reports (109-16). This process, as reported above, gives conversions approaching 90% of theoretical.

The levo form of the glycol has been found to have value as a permanent antifreeze, but the meso form is not satisfactory for this use because of the several hydrates it yields (117-119).

9. **Citric, Gluconic, and Itaconic Acids.** Citric acid is made by fermentation of sucrose solutions by *Aspergillus niger* and the methods used are described in a recent review by Von Loesecke (120). Karow and Waksman (121) have found that by use of a proper mineral balance and with oxygen or air under pressure, a selected strain of *Aspergillus wentii* can be used successfully in submerged culture production of citric acid from sucrose, glucose or cane molasses substrates.

Williams (122) has described the commercial production of gluconic acid by *Aspergillus niger* from 10% glucose medium with aeration at 30°C. The mycelium is used to inoculate a subsequent fermentation. The yield of gluconic acid is reported to be 95% by weight of the glucose processed.

The production of itaconic acid on small commercial scale was started in 1947. Optimum conditions are described by Lockwood and associates (123-125). *Aspergillus terreus* is grown in submerged culture in a substrate containing sugar, magnesium sulfate and corn steep liquor, adjusted to a reaction of pH 1.8 to 2.0.

10. Penicillin and Other Antibiotics. Pasteur noted in 1877 that certain airborne contaminants inhibited the growth of the anthrax bacillus and there was a great deal of research for such antibiotics in the 25 years that followed, but no practical results developed. Fleming noted the antibotic activity of a species of *Penicillium* toward *Staphylococcus aureus* and discovered that this was due to a chemical produced by the mold. Although he did not succeed in isolating the pure compound, he gave it is name "penicillin." A group of bacteriologists and chemists at Oxford University in 1937 found that penicillin surpassed sulfonamides in effectiveness against many pathogens and further that it is almost non-toxic toward animals. In 1941 Florey, director of the Oxford program, was sent to the United States to promote interest in this project.

A cooperative research program in which government, university and industrial laboratories participated resulted, with Coghill and associates at the Northern Regional Research Laboratory of the U. S. Department of Agriculture serving as the coordinating agency. Some of the accomplishments are described by Coghill (126). Out of this program came the large and rapidly expanding production of penicillin in this and other countries.

Many thousands of strains of *Penicillium* have been tested and several have been found that yield higher concentrations of penicillin than are obtained with Fleming's original culture of *Penicillium notatum*. A number of cultural practices have been tested. The first commercial production used shallow surface culture on liquid media. Then it was found that certain strains could be grown submerged in a medium containing corn steep liquor. Attempts to produce penicillin by growth of the mold on wheat bran have not been successful but spore inoculum is regularly developed on such a substrate. Submerged culture in a medium containing glucose, corn steep liquor and other nutrients, with good aeration, is now the general practice.

Antibiotics from other microorganisms are receiving considerable attention in research. Streptomycin from *streptomyces griseus* and tyrothricin from *Bacillus brevies* are examples of products that seem to have desirable properties. Many others are receiving intensive study. Stefaniak *et al.* (127) have described a pilot plant for research on antibiotics production.

11. Other Fermentation Products. It has been the objective in this review to consider those fermentation processes now used to produce useful chemicals from starch or from glucose. No attempt has been made to describe the many other fermentation products that have not yet been carried beyond the laboratory stage. The use of *Acetobacter suboxydans* for the manufacture of sorbose, dihydroxyacetone and other interesting products (128-31), the acetone-ethanol fermentation of starch (132-33), the production of propionic acid (134-136). and many other processes will undoubtedly receive attention in the future. The many reports by Raistrick (137-138) on the metabolic products of mold growth are a mine of information that will attract research attention to new fermentation chemicals.

The availability of starch in large tonnage as a byproduct of a progressive and efficient agriculture, the decreasing supplies of petroleum, and the limitations in the production of blackstrap molasses, coupled with the continually expanding consumer demand for commodities made from fermentation chemicals, clearly indicate that research should actively continue in this field. Interesting commercial developments in the immediate future seem definitely in order.

BIBLIOGRAPHY

1. Fulmer, E. I., and Werkman, C. H., The Chemical Action of Microorganisms, Charles C. Thomas, Baltimore (1930).
2. Groggins, P. H., Gabriel, C. L., Christensen, L. M., Burger, E. K., and Schroeder, W. C., a Five Author Symposium, *Chem. Eng. Progress,* **44**, 13-17 (1948).
3. Boruff, C. S., and Van Lanen, J. M., *Ind. Eng. Chem.,* **39**, 934 (1947).
4. Rubber Reserve Company, Report on the Rubber Program 1940-45 (1945).
5. Jacobs, P. B., *Chemurgic Digest,* **7**, No. 5, 11 (1948).
6. Anon, *Chem. and Met. Eng.,* **52**, No. 11, 114 (1945).
7. Hale, W. J., Farmward March, Coward-McCann, New York (1939).
8. Christensen, L. M., Hixon, R. M., and Fulmer, E. I., Power Alcohol and Farm Relief, Chemical Foundation, New York (1934).
9. Shepherd, G., McPherson, W. K., Brown, L. T., and Hixon, R. M., *Iowa Corn Research Inst.,* **1**, No. 3, June (1940).
10. Willkie, H. F., and Kolachov, P. J., Food for Thought, Indiana Farm Bureau, Indianapolis (1942).
11. Filley, H. C., Loeffel, W. J., and Christensen, L. M., Nebraska Chemurgy Prog. Rept. No. 9 (1943).
12. Hilbert, G. E., *Chemurgic Digest,* **7**, No. 5, 8 (1948).
13. Colwell, A. T., Cummings, R. E., and Anderson, D. E., Alcohol-Water Injection, scheduled for presentation before Soc. Auto. Engrs., Jan. 8, 1945.
14. Brooks, D. B., Nat'l Bur. Stds. Res. Paper RP 1660, *J. Research Natl. Bur. Standards,* **35**, July, (1945).
15. Wetzstein, H. L., *Chemurgic Digest,* **7**, No. 4, 24 (1948).
16. Waksman, S. A., and Davison, W. C., Enzymes, Williams and Wilkins, Baltimore, 268 (1926).
17. Langford, C. T., *Chemurgic Digest,* **7**, No. 5. 4 (1948).
18. Beresford, H., and Christensen, L. M., *Idaho Agr. Expt. Sta. Bull.* No. **241** (1941).
19. Christensen, L. M., *Cereal Chem.,* **20**, 478 (1943).
20. Christensen, L. M., U. S. Patent 2,342,330 (1944) and U. S. Patent 2,304,679 (1942).
21. Underkofler, L. A., unpublished data (1945).

22. Christensen, L. M., and Taylor, G. T., U. S. Patent Application 438,842 (1942).
23. Christensen, L. M., U. S. Patent 2,348,451 (1944).
24. Severson, G. M., Kneen, E., and Lewis, K. H., U. S. Patent Application 653,433 (1946).
25. Beckord, L. D., Kneen, E., and Lewis, K. H., *Ind. Eng. Chem.*, **37**, 692 (1945).
26. Beckord, L. D., Peltier, G. L., and Kneen, E., *Ind. Eng. Chem.*, **38**, 232 (1946).
27. Grove, O., *J. Inst. Brewing*, **20**, 248 (1914).
28. Owen, W. L., *Ind. Eng. Chem.*, **25**, 87 (1933).
29. Severson, G. M., *Iowa State Coll. J. Sci.*, **11**, 215 (1937).
30. Underkofler, L. A., Severson, G. M., Goering, K. J., and Christensen, L. M., *Cereal Chem.*, **24**, 1 (1947).
31. Underkofler, L. A., Severson, G. M., and Goering, K. J., *Ind. Eng. Chem.*, **38**, 980 (1946).
32. Erb, N. M., and Hildebrandt, F. M., *Ind. Eng. Chem.*, **38**, 792 (1946).
33. Adams, S. L., Balankura, B., Andreasen, A. A., and Stark, W. H., Improvements in Methods for Submerged Culture of Fungal Amylase, paper read before ACS meeting April 14-17 (1947).
34. Back, T. M., Stark, W. H., and Vernon, C. C., *Ind. Eng. Chem.*, **40**, 80 (1948).
35. Underkofler, L. A., and Wimmer, E. L., Unpublished data (1946).
36. Kneen, E., and Sandstedt, R. M., *Arch. Biochem.*, **9**, 235 (1945).
37. Miller, B. S., and Kneen, E., *Arch. Biochem.*, **15**, 251 (1947).
38. Christensen, L. M., and Taylor, G. T., Unpublished data (1948).
39. Perkin, W. H., *J. Soc. Chem. Ind. (London)*, **31**, 616 (1912).
40. Gill, A., *J. Soc. Chem. Ind. (London)*, **38**, M3T (1919).
41. Killefer, O. H., *Ind. Eng. Chem.*, **19**, 46 (1927).
42. Gabriel, C. L., *Ind. Eng. Chem.*, **20**, 1063 (1928).
43. Gabriel, C. L., and Crawford, F. M., *Ind. Eng. Chem.*, **22**, 1163 (1930).
44. Wynkoop, R., *Ind. Eng. Chem.*, **35**, 1240 (1943).
45. Severson, G. M., and Christensen, L. M., Unpublished data (1934).
46. Severson, G. M., Thesis, Iowa State College, Ames (1936).
47. Christensen, L. M., Unpublished data (1929).
48. Johnson, M. L., Peterson, W. H., and Fred, E. B., *J. Biol. Chem.*, **91**, 569 (1931).
49. Legg, D. A., U. S. Patent 1,668,814 (1928).
50. Starr, D. F., Thesis, Iowa State College, Ames (1933).
51. Legg, D. A., and Stiles, H. R., U. S. Patent 2,089,562 (1937).
52. McCoy, E. F., U. S. Patent 2,110,109 (1938).
53. Arzberger, C. F., U. S. Patent 2,139,108 (1938).
54. Hall, H. E., U. S. Patent 2,147,487 (1939).
55. Hildebrandt, F. M., and Erb, N. M., U. S. Patent 2,169,246 (1939).
56. Bryner, L. C., Christensen, L. M., and Fulmer, E. I., *Ind. Eng. Chem.*, **28**, 206 (1936).
57. Underkofler, L. A., Christensen, L. M., and Fulmer, E. I., *Ind. Eng. Chem.*, **28** 350 (1936).
58. Underkofler, L. A., Fulmer, E. I., and Rayman, M. M., *Ind. Eng. Chem.*, **29**, 1290 (1937).
59. Dunning, J. W., and Lathrop, E. C., *Ind. Eng. Chem.*, **37**, 24 (1945)
60. Neuberg, C., and Hildescheimer, A., *Biochem. Z.*, **31**, 170 (1911)
61. Neuberg, C., and Reinfurth, E., *Biochem. Z.*, **106**, 281 (1920)
62. Connstein, W., and Ludecke, W., U. S. Patent 1,511,754 (1924)

63. Cocking, A. T., and Lilly, C. H., U. S. Patent 1,425,838 (1922).
64. Eoff, J. R., U. S. Patent 1,288,398 (1918).
65. Eoff, J. R., Linder, W. V., and Beyer, G. F., *Ind. Eng. Chem.*, **11**, 842 (1919).
66. Ling, A. R., *J. Soc. Chem. Ind.*, **38**, 175 (1919).
67. May, O. E., and Herrick, H. T., *Ind. Eng. Chem*, **22**, 1172 (1930).
68. Nord, F. F., *Chem. Revs.*, **26**, 423 (1940).
69. Underkofler, L. A., Hickey, R. J., and Fulmer, E. I., U. S. Patent 2,388,840 (1945).
70. Underkofler, L. A., Hickey, R. J., and Fulmer, E. I., U. S. Patent 2,416,745 (1947).
71. Smith, L. T., and Claborn, H. V., *Ind. Eng. Chem. News Ed.*, **17**, 370 (1939).
72. Claborn, H. V., and Smith, L. T., *J Am. Chem. Soc.*, **61**, 2727 (1939).
73. Smith, L. T., and Claborn, H. V., *Ind. Eng. Chem.*, **32**, 692 (1940).
74. Smith, L. T., Fisher, C. H., Ratchford, W. P., and Fein, M. L., *Ind. Eng. Chem.*, **34**, 473 (1942).
75. Mast, W. C., Smith, L. T., and Fisher, C. H., *Ind. Eng. Chem.*, **37**, 365 (1945).
76. Ratchford, W. P., and Fisher, C. H., *Ind. Eng. Chem.*, **37**, 382 (1945).
77. Filachione, E. M., Lengel, J. H., and Fisher, C. H., *Ind. Eng. Chem.*, **37**, 388 (1945).
78. Peckham, G. T., Jr., *Chem. Eng. News*, **22**, 440 (1944).
79. Jenemann, J. A., U. S. Patent 1,906,068 (1933).
80. Dietz, A. A., Degering, E. F., and Schopmeyer, H. H., *Ind. Eng. Chem.*, **39**, 82 (1947).
81. Breden, C. R., and Fulmer, E. I., *Iowa State Coll. J. Sci.*, **5**, 133 (1931).
82. Fulmer, E. I., Christensen, L. M., and Kendall, A. R., *Ind. Eng. Chem.*, **25**, 798 (1933).
83. Kluyver, A. J., and Scheffer, C., U. S. Patent 1,899,156 (1933).
84. Christensen, L. M., U. S. Patent 2,344,025, March 14, 1944.
85. Hill, R., and Isaacs, E., U. S. Patent 2,224,912 (1940).
86. Elder, A. L., *Ind. Eng. Chem.*, **34**, 1260 (1942).
87. Ward, G. E., Pettijohn, O. G., Lockwood, L. B., and Coghill, R. D., *J. Am. Chem. Soc.*, **66**, 541 (1944).
88. Ward, G. E., Pettijohn, O. G., and Coghill, R. D., *Ind. Eng. Chem.*, **37**, 1189 (1945).
89. Ward, G. E., Wickerham, L. J., Pettijohn, O. G., and Lockwood, L. B., U. S. Patent 2,359,950 (1944).
90. Katznelson, H., *Can. J. Research*, **22c**, 235 (1944).
91. Katznelson, H., *Can. J. Research*, **22c**, 241 (1944).
92. Katznelson, H., and Lockhead, A. G., *Can. J. Research*, **220**, 273 (1944).
93. Ledingham, G. A., and Adams, G. A., *Can. Chem. Process Inds.*, **28**, 742 (1944).
94. Neish, A. C., *Can. J. Research*, **23B**, 10 (1945).
95. Rose, D., and King, W. S., *Can. J. Research*, **23F**, 79 (1945).
96. Ledingham, G. A., Adams, G. A., and Stanier, R. Y., *Can. J. Research*, **23F**, 48 (1945).
97. Stanier, R. Y., Adams, G. A., and Ledingham, G. A., *Can. J. Research*, **23F** 72 (1945).
98. Adams, G. A., and Stanier, R. Y., *Can. J. Research*, **23B**, 1 (1945).
99. Adams, G. A., and Leslie, J. O., *Can. J. Research*, **24F**, 107 (1946).
100. Katznelson, H., *Can. J. Research*, **24c**, 99 (1946).
101. Neish, A. C., *Am. Perfumer Essent Oil Rev.*, **48**, 59 (1946).
102. Neish, A. C., Blackwood, A. C., and Ledingham, G. A., *Can. J. Research*, **23B**, 290 (1945); *Science*, **101**, 245 (1945).
103. Adams, G. A., *Biochem. J.*, **38**, 168 (1944).

104. Strohmaier, A. J., and Lovell, C. L., *Ind. Eng. Chem.*, **38**, 721 (1946).
105. Walmsley, R. A., and Davis, U. R., U. S. Patent 2,397,065 (1946).
106. Cornwell, R. T. K., U. S. Patent 2,390,779 (1945).
107. Senkus, M., *Ind. Eng. Chem.*, **38**, 913 (1946).
108. Kolfenbach, J. J.,, *Iowa State Coll. J. Sci.*, **19**, 35 (1944).
109. Blom, R. H., Reed, D. L., Efron, A., and Mustakas, G. C., *Ind. Eng. Chem.*, **37** 870 (1945).
110. Schniepp, L. E., Dunning, J. W., and Lathrop, E. C., *Ind. Eng. Chem.*, **37**, 87 (1945).
111. Morell, S. A., Geller, H. H., and Lathrop, E. C., *Ind. Eng. Chem.*, **37**, 877 (194
112. Schniepp, L. E., Dunning, J. W., Geller, H. H., Morell, S. A., and Lathrop, E. C *Ind. Eng. Chem.*, **37**, 884 (1945).
113. Othmer, D. F., Bergen, W. S., Shlechter, N., and Bruins, P., *Ind. Eng. Chem.*, **37**, 890 (1945).
114. Othmer, D. F., Shlechter, N., and Koszalka, W. A., *Ind. Eng. Chem.*, **37**, 895 (194
115. Shlechter, N., Othmer, D. F., and Marshak, S., *Ind. Eng. Chem.*, **37**, 900 (1945).
116. Shlechter, N., Othmer, D. F., and Brand, R., *Ind. Eng. Chem.*, **37**, 905 (1945).
117. Coghill, E. D., and Milner, R. T., U. S. Patent 2,369,435 (1945).
118. Clendenning, K. A., *Can. J. Research*, **24F**, 249 (1946).
119. Clendenning, K. A., and Wright, D. E., *Can. J. Research*, **24F**, 287 (1946).
120. Von Loesecke, H. W., *Chem. Eng. News*, **23**, 1952 (1945).
121. Karow, E. O., and Waksman, S. A., *Ind. Eng. Chem.*, **39**, 821 (1947).
122. Williams, A. E., *Mfg. Chemist*, **16**, 239 (1945).
123. Lockwood, L. B., and Ward, G. E., *Ind. Eng. Chem.*, **37**, 405 (1945).
124. Lockwood, L. B., and Reeves, M. D., *Arch. Biochem.*, **6**, 455 (1945).
125. Lockwood, L. B., and Nelson, G. E. N., *Arch. Biochem.*, **10**, 365 (1946).
126. Coghill, R. D., *Chem. Eng. News*, **22**, 588 (1944).
127. Stefaniak, J. J., Gailey, F. B., Brown, C. J., and Johnson, M. J., *Ind. Eng. Chem* **38**, 666 (1946).
128. Fulmer, E. I., Dunning, J. W., Guymon, J. F., and Underkofler, L. A., *J. Am Chem. Soc.*, **58**, 1012 (1936).
129. Wells, P. A., Lockwood, L. B., Stubbs, J. J., Roe, E. T., Perges, N., and Gastrock E. A., *Ind. Eng. Chem*, **31**, 1518 (1939)
130. Neuberg, C., and Hofman, E., *Biochem. Z.*, **279**, 318 (1935).
131. Underkofler, L. A., and Fulmer, E. I., *J. Am. Chem. Soc.*, **59**, 301 (1937).
132. Northrop, J. H., Ashe, L. H., and Senior, J. K., *J. Biol. Chem.*, **39**, 1 (1919)
133. Fred, E. B., Peterson, W. H., and Anderson, J. A., *Ind. Eng. Chem.*, **15**, 126 (1923)
134. Whittier, E. O., and Sherman, J. M., *Ind. Eng. Chem.*, **15**, 729 (1923).
135. Werkman, C. H., Hixon, R. M., Fulmer, E. I., and Rayburn, C. N., *Proc. Io Acad. Sci.*, **36**, 111 (1929).
136. Wilson, P. W., U. S. Patent 1,898,329 (1933).
137. Raistrick, H., *Trans. Roy. Soc. (London)*, **B220**, 1-367 (1931).
138. Raistrick, H., *Biochem. J.*, from 1931 to date.

Chapter XX

USE OF STARCH AND STARCH PRODUCTS IN FOODS

1. **Introduction and General Discussion.** The food industries comprise one of the largest consumers of starch and starch products.* In addition, about one-sixth of all starch sold for food purposes is purchased by the housewife in the form of small packaged products for domestic cooking. Not only are purified and treated starches used for such varied purposes as starch puddings, confections, salad dressings, pies, and other bakery products but products derived from starch such as sirups and refined sugar are used to a large extent in a host of additional foods as well, such as ices, ice cream, cake and bread. The manufacture of baking powder consumes a large portion of the starch sold for food purposes. Furthermore, as is generally known, a very considerable tonnage of dry milled flour is used for baking bread, cake, muffins, and similar foods. These flours include those of wheat, rye, corn, and buckwheat. Wheat flour is used extensively in the United States.

Starch and derived sugars provide a large proportion of our caloric requirements. They are energy foods, and the dietary function is the same whether the pure starch is added as such to thicken a soup or a pie filling or to produce a delectable pudding, or eaten in its natural association with other food elements such as the flour used to make bread. One pound of starch provides 1800 calories, which would amount to 50% of the daily energy requirements of a hard working laborer. It is evident that the three hundred million pounds of pure starch utilized each year for food purposes in America, provide more than a negligible portion of the nation's calorific requirements. One of the principal reasons for including pure starch in food products is the effect it has on the texture or consistency of the prepared food. Accordingly, its contribution to the dietary requirements may be overlooked by the consumer.

Starches used extensively for food prior to 1943 were, corn, tapioca, potato, wheat, rice, sago, and arrowroot. With the disruption in world trade which followed, particularly the cessation of exports from the Dutch West Indies, the amount of tapioca, sago and other tropical starches used declined to an almost insignificant value. During this period the waxy starches, such as maize and sorghum were studied extensively and developed in order to provide a replacement for normal non-cereal starches. For example, pearl tapioca, sold for making a characteristic pudding dessert virtually disappeared from the market. Attempts to make a similar product from corn and other available starches were largely unsuccessful and considerable effort was expended in utilizing the waxy starches for this purpose. Research during recent years has produced modifications of available starches which are in some respects superior to the natural, imported starches, as well, of course, to the run-of-the-mill domestic starches which were used extensively for food purposes in former years.

---

*Of 1.5 billion pounds of corn starch sold annually in the U.S.A., food industries consume 350 millions in bulk. Approximately 75 million pounds are sold in small packages, also for food use.

In foods, particularly as used in pie fillings, puddings, soups and sauces, both in domestic cooking and in large commercial operations, the colloidal properties of a particular starch product as it affects the texture, taste, and appearance of the prepared food is the determining factor in its selection. Price, in general, is secondary. It is not unusual for bakeries, for example, to pay a premium of 50% over the base price for the most available starch, in order to use some particular type or modification best suited for making pies.

In the United States of America, of the three million or more pounds of starch consumed annually in foods, nearly 90% is corn or modified corn starch. Of this, the more important uses, from a volume standpoint are, in million pounds, brewers, 113; baking powder manufacturers, 63; confectioners, 30; and bakers 27. The amount sold in packages for diversified domestic use is about fifty million pounds. About ten million pounds is sold in the form of prepared dessert mixtures. Besides this amount of milled starch, a very much larger amount is consumed as flour by the baking trade, as cereal products in prepared foods and in the production of edible fermentation products.

Inasmuch as the use of starch and starch products is of a very diversified nature in respect to foodstuffs, no attempt will be made to discuss each particular application. In the following sections several of the representative uses of both starch and flours will be outlined. The reader is referred to another chapter in this text for a discussion of the utilization of starch products in fermentation.

2. **Flour in Bread Making.** Although starch is the major constituent of flours, other constituents of the grain are also present, in smaller amounts, which enhance its use for some purposes. Wheat flour, with which this discussion is chiefly concerned, contains about 72 to 75% of starch, 7 to 13% of protein, 0.4 to 0.7% of ash, and small amounts of natural sugars, fats, enzymes, and other organic components. Although the amount of protein present is relatively small compared to the amount of starch present, it exerts a very pronounced influence on the character of products made from flour. Indeed, the art of baking breadstuffs depends to a large measure on the selection of flour with the proper gluten characteristics and the correct

transformation and utilization of this constitutent before and during bread making.) 6

The ability of flour to swell in cold water or milk and form a dough depends principally on the gluten present. Starch does not swell in cold water (except to a very insignificant extent and except for a few granules which may have been injured in the milling process). The swelling and water absorption which occur when pure starch is stirred in water at the temperature of the mixing of dough and fermentation result in a granular, non-coherent mass that in no way resembles the plastic dough that results when flour is hydrated. Obviously, starch may be made to swell in cold water and to absorb more moisture at lower temperatures by severe overgrinding and thus materially increase the capacity of a flour to hydrate. This special phase of the subject has received due attention not only in respect to the hydration of flour but as a means of making the raw starch more available for enzymic activity in the mixing and fermentation operations.

Leavening of dough is generally brought about by carbon dioxide gas generated in the dough either by an induced fermentation or by the admixture of carbonates which will release carbon dioxide. The discussion in this section is concerned chiefly with some of the problems involved when the former method of leavening is used.

Fermentation in dough is brought about by the action of enzymes in the flour and by those of added yeast (and occasionally malt) on the carbohydrates and proteins in the dough. Flour contains a saccharogenic diastase, β-amylase, and a fairly potent proteinase called papainase, and yeast furnishes maltase and zymase. The latter are involved in the later stages of fermentation in the production of carbon dioxide, alcohol, and characteristic flavoring compounds. Saccharogenic activity is influenced by the amount of soluble carbohydrate present, the susceptibility of the starch to enzymic action, and the amount of active enzyme available. Often these factors are inadequate for a flour to supply the yeast with sufficient sugar for a normal fermentation. To correct this, such adjuncts may be added as malt preparations, sirups, and sugars. The action of the proteinase must be controlled, for its action, if permitted, produces undesirable results in the baking quality of the dough. Enzymes of the papain type are readily inactivated by oxidation. Hence a pretreatment of the flour, *e.g.*, by added oxidants, atmospheric oxidation during storage, or the addition of oxidizing reagents to the dough, will inactivate this enzyme. Finally, the metabolic state of the yeast employed is also important (1).

The baking properties of flour are expressed in terms of the behavior of the dough in the bakery. Such characteristics are as follows: strength, stability, fermentation tolerance, tolerance to mechanical treatment, and tolerance to oxidizing agents. These have been ably discussed by Blish (2). Strength is the capacity for making a loaf of large volume per unit weight and is principally a function of the protein present. Stability is the resistance a dough offers to becoming tacky or sticky during fermentation. Fermentation tolerance is related to the length of the time interval over which good bread can be kept in a state of fermentation and is dependent on the "gassing power" of the dough. Mechanical tolerance indicates the degree of stability of the dough in mixing and kneading operations. Tolerance to oxidizing agents, when they are added, is a very important property. Differences in the responses shown by different flours necessitate careful control in the use of oxidizing agents by the miller or by the baker in order to secure uniformity in the final baked product. The quality of the baked bread is measured by its volume per unit weight, its texture, its moisture content, the character of its crust, and its keeping quality.

The so called "diastatic power" of a flour, that is, its ability to furnish the added yeast with fermentable carbohydrate, depends upon (*a*) the amylase content of the flour, (*b*) the nature of the amylases, (*c*) their availablity or activation, and (*d*) the nature of the original carbohydrate in the flour.

The amylase content of flour varies considerably with the source. Geddes and Eva (3) found that 80 flours varied in diastatic activity from approximately 70 to 300 units. The enzyme constituent present is principally β-amylase and, as is the case in most resting grains, it appears to be intimately bound with other constituents and is not readily liberated. Although dilute salt solutions are effective in releasing the inactive or bound enzyme in wheat, as has been shown by Sandstedt *et al.* (4), it is not likely that the amounts present in dough are very effective in such liberation. The grain is naturally provided, apparently, with a means for releasing enzyme. Ford and Guthrie (5) showed many years ago that a treatment of barley with the protein-splitting enzyme, papain, is effective in the release of barley amylase. They also called attention to the solubilizing effect of salt. Józsa and Gore (6) found that treatment with papain greatly increases the solubility of the amylase in wheat flour. Apparently, the amylase is attached in some manner to the gluten. Although wheat contains a papainase, unfortunately the activity of this protein-splitting enzyme cannot be permitted to exert itself in dough. Therefore, many studies have been made to determine and overcome the shortcomings of wheat flours in respect to their diastase content. Stamberg and Bailey (7) have recently published some interesting results which more or less summarize the problem. These investigators added purified enzyme preparations to flour; the enzymes added were β-amylase, which is present in flour at least in combined form, and α-amylase, which, if present at all, is in ~~a~~ more inactive state than the β component. 7 Their experiments indicate that normal flour does not require the addition of β-amylase, since the flour is not materially improved by such addition. However, the addition of small quantities of α-amylase, the starch-liquefying or dextrinizing component of malt diastase, brought about a marked improvement of the volume of the loaf at various fermentation times for the dough. Some of the results of their baking experiments are shown in Fig. ~~103~~. These results support the contention of Blish *et al.* (8), that the α-amylase is the more important enzyme and that β-amylase has very little effect on the diastatic activity of dough made from normal flour. Sandstedt *et al.* (9), believe that the improvement effected by adding malt to flour is brought about by the α-amylase content of the malt diastase. Not all flours respond equally well to the added α-amylase. From the view-point of starch chemistry the above results might be interpreted to mean that the inability of a dough to supply yeast with sufficient fermentable sugar is not so much due to the fact that a large share of the β-amylase is in an inactive state and that there exists a scarcity of this factor, but rather that it is due to a scarcity of suitable substrate for the β-amylase in normal flour. The presence of a starch-liquefying component is required. The latter is apparently better able to function on the native carbohydrate than is the β-amylase.

fig 8

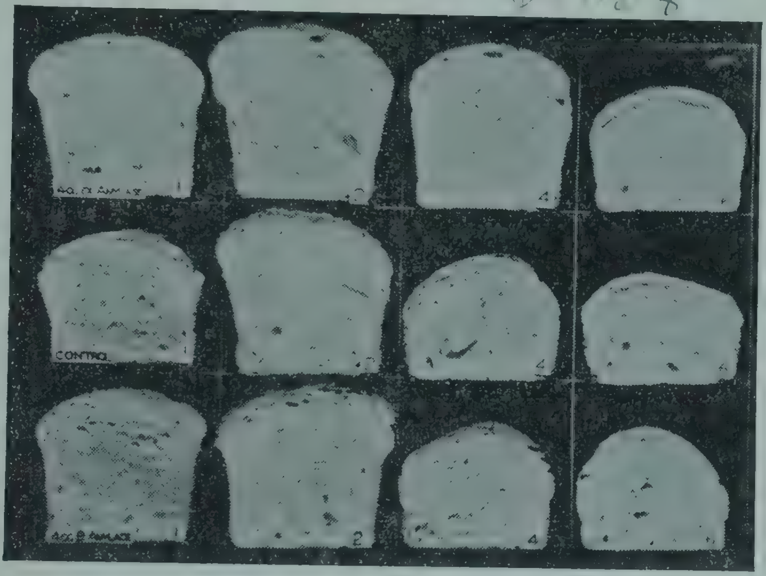


FIG. 103. Interior of bread made with additions of α- and β-amylase in doughs with 1, 2, 4, and 6 hrs. of fermentation. Upper row, 4 cc. of α-amylase added; middle row, control; lower row, 4 cc. of β-amylase added.

The question of injured granules cannot, of course, be overlooked in this discussion. Early workers, for example Brown and Heron (10) and Maquenne (11), observed that mechanically injured starch granules are more readily attacked than sound granules. The writer has observed this effect with special reference to starch-liquefying enzymes, using prolonged ball milling of corn starch to effect the mechanical injury. It is obvious, therefore, that a flour so milled as to disorganize the enzyme-resistant orientation of even a small proportion of the starch granules present will exhibit an increased fermentation rate, at least in the initial stages. It is this early stage of dough fermentation on which the focus of attention of most investigators is fixed for perfectly understandable reasons. Hard wheats, which require comparatively strenuous grinding, should therefore possess more injured starch granules and, other things being equal, respond more favorably to the action of added diastase than flour from softer wheats. Collatz (12) found, in accordance with the above theory, that the starch of strong flours appears to be more easily hydrolyzed than that of weaker flours. Alsberg (13) has suggested that flours which are weak in "diastatic power" might be overground to correct this shortcoming. This investigator also suggested the addition of small quantities of severely overground flours to those with deficient "diastatic power" in order to improve them.

The difference in the response of various flours is probably not so simply explained, however, as indicated above. It is quite likely that the character of the gluten and the physical association of the starch and gluten play an important role as well. In soft or "floury" wheats, as in other "floury" grains, much of the starch is free, whereas in hard or "horny" grains, the starch is embedded in a matrix of gluten which condition not only limits the accessibility of the starch to the enzyme, but also tends to change the character of the starch as well.*

An alternate solution to the problem just discussed, obviously, is to furnish the yeast with a ready supply of fermentable carbohydrate. It is apparent, of course, that acceleration of dough fermentation is very desirable from an industrial point of view, since production is speeded up accordingly. Furnishing an active yeast with a ready supply of fermentable material and thus dispensing with the intermediate phase of diastatic activity would seem to be the logical method of best contributing to this end. Many relatively inexpensive sources of carbohydrate are available in the forms of sirup and sugar. Not all sirups, however, possess high fermentability. Malt sirup does contain a high percentage of fermentable solids and may be added for this purpose. According to Rumsey (14) some thirty million pounds of malt extract have been used annually by American bakers in the past. But since malt sirup contains a very plentiful supply of diastatic enzymes, as well as available carbohydrate, the danger arises that the dough may be oversupplied with diastase. Stamberg and Bailey (7) point out that sogginess of crumb may result from adding too much diatase to the dough and suggest that many of the poor baking results in the past may be traced to this factor.

---

*Refer to the sections on corn starch in Chapters I and II for further comparisons of "floury" and "horny" starch.

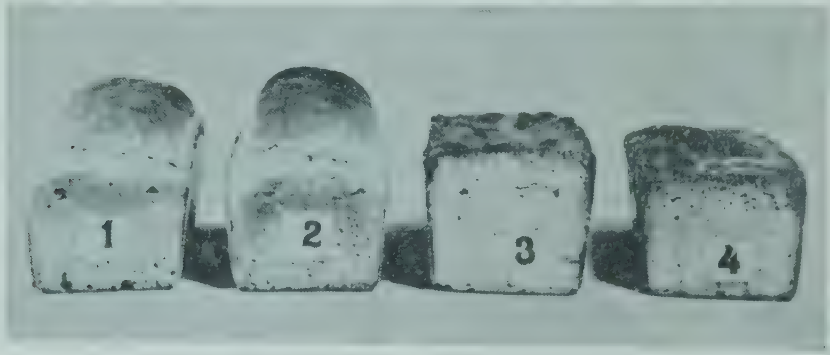
The fermentability of the relatively less expensive and as easily handled sirups from corn starch has recently been investigated by Kerr and Schink (15). Some of these types, such as for example the "Corn Sirup, Unmixed" (CSU) of commerce, are rather low in the percentage of solids fermentable by yeast. They contain usually less than 50% of fermentable solids. Kerr and Schink point out that several concerns have developed newer types of corn sirups made by processes involving a diastatic treatment. These sirups are of the order of 65 to 70% fermentable. A sirup such as described in the patent of Dale and Langlois (16) is an example. More recently Kerr *et al.* (17) disclosed methods for producing very highly fermentable, stable corn sirups, with fermentable solids in the range of 80 to 85%. The latter should, accordingly, be very near the ultimate for those industries which prefer a readily available source of fermentable carbohydrate in liquid form.

Of course the peak in fermentability is the pure sugar, glucose or dextrose as it is more commonly referred to in industry. Dextrose is theoretically 100% fermentable. Although cane sugar is fermented by yeast, it requires a hydrolysis to its simple sugar constituents, dextrose and fructose, by the enzyme invertase before the fermentation reactions proper begin. The development of the manufacture of pure, crystalline dextrose in the United States during the last two decades has been a great boon to the baking industry, among others. Its use was readily adopted by the baking trade as an additive to doughs to furnish the yeast with the required amount of fermentable substance to produce an excellent bread. Today the baking trade is one of the largest consumers of this product, derived from starch, the production of which now requires probably a quarter of all the corn starch milled in the United States.

4 The advantage of dextrose to the baker is not only that it is quickly and completely fermentable but also that it improves the quality of the bread in several respects. The golden brown color of the crust and its keeping quality after baking are two such qualities which may be mentioned, which result from the addition of dextrose in slight excess of the actual requirements of the yeast.) 12

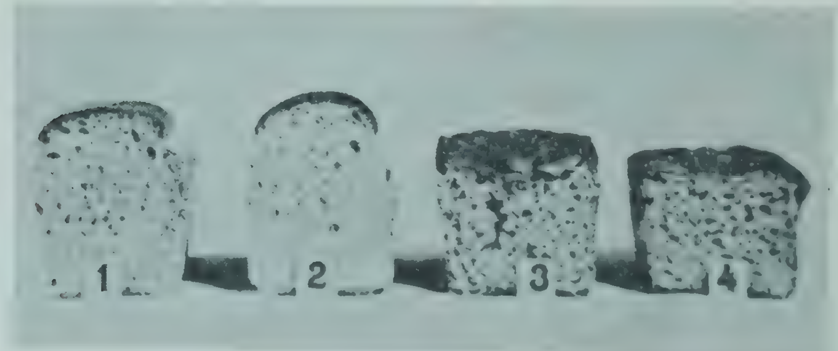
All baking aids or adjuncts are not necessarily concerned with carbohydrate modification or utilization in dough or bread making. Some aids are considered as yeast foods or stimulants, while others are useful in inactivating the proteinase of wheat flour, which enzyme exerts a deleterious effect on the wheat gluten. Balls and Hale (18) and Jorgensen (19) have shown that wheat proteinase is a papain-like enzyme. It is activated by reduced glutathione and is inactivated by oxidizing agents such as are present in some bread improvers. Potassium bromate, for example, inactivates the proteinase of wheat flour. These enzymes act first to produce a clot with the flour proteins and then to produce a liquefying action so that the dough loses its strength or ability to retain the carbon dioxide of fermentation. Little or no raising of the dough results in extreme cases.

Balls and Hale (20) carried out baking experiments in which potassium persulfate was added to inactivate the proteinase. Similar loaves were made which included papain and glutathione instead of the persulfate. The results of these baking tests are shown in Figs. 104 and 105. The photographs of



Courtesy of Cereal Chemistry

FIG. 104. Exterior of 1 lb. loaves of bread containing: Loaf 1, no addition (control); Loaf 2, 50 mg. of potassium persulfate; Loaf 3, 50 mg. of papain; Loaf 4, 50 mg. of glutathione.



Courtesy of Cereal Chemistry

FIG. 105. Interior of loaves of bread shown in Fig. 104.

the baked loaves clearly illustrate the value of destroying the proteinase and the adverse results obtained when the papain activity is increased.

The aging of flour, either naturally or as induced by chemicals, has long been known to improve its strength. It now appears that the action is not so much a change in the physical properties of the gluten or the starch, as it is rather, the increased strength which results from such action due to a reduction in the proteinase activity of the flour. This view has not, however, been universally accepted in the past. Some workers have maintained that the action of oxidizing agents is primarily on the protein and have, indeed, proposed a chemical mechanism, that of action on the sulfhydryl groups. Others have accepted a compromise theory that the action of some proteinases on wheat gluten is very much the same as the action of reducing substances and that the addition of oxidizing agents inactivates or destroys both (21-26). According to one view, gluten exists naturally in the "reduced" state, which is the weaker, and is converted into the stronger state by oxidants, although the process is reversible.

Elion (27) has shown, however, that when untreated wheat flour was heated for the purpose of destroying the naturally occurring enzymes, the addition of 16% mg. of Na_2SO_3 (which materially increased the softening of dough made from unheated flour) had practically no effect on dough made from the heated flour, whereas, the addition of papain, and particularly papain plus the Na_2SO_3 materially softened dough made from the heated flour. Thus, the action of reducing substances is not the same as that of papain but rather, is a secondary one of activating the enzyme.

Furthermore, since the heated flour formed a dough which did not soften with age, one may conclude either that the enzyme system which acts on the gluten was destroyed or that the prolonged heat treatment induced an atmospheric oxidation during which the weak "reduced" form of gluten was changed to the oxidized. Geddes (28), Kent-Jones (29) and others have shown that oxidants such as $KBrO_3$ do not improve heat-treated flours. However, if this change is so readily reversible as proponents of the oxidation-reduction theory have assumed, then the addition of reducing substances alone should have softened the dough from the heat-treated flour. Since the addition of papain was also required, it appears logical to conclude with Elion that present data can most satisfactorily be interpreted on the basis of an enzymic reaction.

For a full description of baking tests and for a discussion of various factors and variables which influence the results, the treatment of which is outside the scope of this chapter, reference is made to the reports of Blish (30), Markley and Bailey (31), Geddes *et al.* (32), Schultz and Landis (33), Landis and Frey (1), and the report of Kent-Jones mentioned above. Reference is also made to a report of Swanson and Johnson (34) relating to the use of a recording dough mixer in the study of the quality of flour for baking.

An important factor to be considered in bread is its keeping quality after baking. Carbohydrate chemists have frequently sought to show a relationship between the modification of the starch in bread making and the staling of bread which follows. The many researches on the subject by Katz and coworkers have been summarized by Katz (35) and therefore only the salient points of his work will be reviewed. The exact interpretation of his theories has been changed by some and these theories have been questioned by others. The essence of what Katz sought to confirm, that the change which occurs within a partially gelatinized starch granule as it cools and ages is closely associated with a change in water relationships and is an essential part of the staling process, still remains to be conclusively disproved by experiment.

13 The obvious change in bread as it stales is that its texture changes from soft or tender to a hard, granular state. The bread loses its characteristic cohesiveness and becomes crumbly. Katz confirmed the reports of early workers and showed that this change was not merely one of dehydration. In a preliminary examination of the problem of staling, Katz and Verschaffelt concluded that staling was principally the result of a change in the physical properties of the gelatinized or partly gelatinized starch in the bread. As the change was examined with the aid of the microscope, it was seen that the starch granules became more sharply outlined. Upon closer examination it was found that the granules became doubly contoured and that between the two was what appeared to be a layer of air. Katz then took as criteria for staling (*a*) changes in the hardness of crumb, which was tested with a specially designed penetrometer, (*b*) changes in the swelling power of the crumb, determined by noting the apparent volume of 10 g. of pulverized breadcrumbs when shaken and allowed to stand from 24 to 48 hours under an excess of water, (*c*) changes in the amount of soluble material made by extracting a known weight of bread with cold water, and then filtering and precipitating the solids from the concentrated extract with an excess of alcohol.

Applying these tests to fresh bread and to the same bread 48 hours after baking, Katz found the following representative figures.

	Hardness Penetrometer depth	Swelling power cc.	Soluble per cent
Fresh bread	0.240	52	5.39
Stale bread	0.030	34	2.88

Using the above criteria, Katz attempted to demonstrate that staling is a process induced by a change in temperature. He found that bread kept 48 hours at 60°C. is still fresh by these same tests, whereas bread kept at 0°C. is more stale than when kept at room temperature. But, on the other hand, greatly reduced temperature, —185°C., prevented staling entirely. The point of maximum velocity for staling was found to be —2° to —3°C. A close parallelism is therefore apparent between these results and the effect of temperature on the speed of retrogradation as observed by Maquenne in his classical researches on the subject.

As might be anticipated from the theory, the addition of alkaline substances, such as pyridine and dipropylamine, was found to be effective in the prevention of staling. However, if staling is due to cross-bonding of molecules of starch by the elimination of water, as Katz suggested (or as the modern theory would explain it, by the formation of hydrogen bonds between carbohydrate hydroxyls, with the release of associated water molecules), it is surprising that acids, chloroform, and alkyl halides should have been reported to be without effect. The latter compounds, it would seem, should have accelerated staling, on the assumption that staling involves an orientation of the starch molecules.

Katz then showed that the changes which take place when bread stales are in the same direction as when starch alone is gelatinized at the same starch to water ratio as in bread and when heated to the same temperature, on the average, as that reached within the loaf of bread when it is baked.

Opposed to the view that staling is essentially a retrogradation effect in the starch, three arguments, mainly, have been advanced by more recent workers. It is pointed out that, whereas stale bread is considered more digestible than fresh bread, retrograded starch is less readily attacked by diastase than a freshly cooked starch paste. This argument is based on two false premises. The principal premise should have been stated, that fresh bread is more "indigestible" than stale. This is due to the doughy or soggy character which fresh bread assumes when it becomes moist, as in mastication. This physical condition prevents the ready absorption of enzymes from the saliva and more particularly of the gastric juices. These chunks, therefore, must remain longer in the stomach and induce the effects commonly associated with indigestion. Secondly, the assumed premise is made that the enzymes responsible for the digestion of bread in the stomach and the liquefaction of starch *in vitro* are the same. This is not true. Proteolytic enzymes are concerned principally in action in the stomach. If the bread is digested, it is converted into a slurry due to a liquefaction of the protein, the mass no longer being held together by a binding agent, the protein. The reaction here is acid, and diastases are sensitive to acidity. This is particularly true of starch-liquefying enzymes, and exposure to a slight acidity may be used as a means of "purifying" β-amylase preparations.

It is quite probable that unless the salivary amylase is enclosed in and protected by a more or less impervious mass such as is the case when fresh bread is eaten, very little diastatic activity survives in the stomach.

It is also pointed out that stale bread may be freshened by a moderate reheating, whereas retrograded starch is not redispersed by heating to the same temperature. The argument is based on the observation that, if starch is thoroughly gelatinized and diluted, or *vice versa*, so that the starch is thoroughly dispersed to allow the more linear chains of starch molecules to orient and precipitate, a granular product is formed which cannot be redispersed except at very high temperatures. In bread, however, there is not sufficient water for complete dispersion or even complete gelatinization of the starch. Indeed, Katz pointed out that the water present is sufficient only to permit the initial stages of gelatinization. Starch heated with a corresponding proportion of water merely swells to form a salvy gel in the case of the cereal starches. Quite likely the formation of such a gel involves some orientation or association but it is definitely more complex, involving, no doubt, the more complex configurations within the starch as well as the linear chains. Cross-bonding probably takes place at random points. But it is hardly comparable to the more complete, "two dimensional" orientation of linear chains with each other, which follows when starch is completely dispersed in dilute solution. Although it is possible that the retrogradation effects in these partially gelatinized starch gels cannot be completely reversed by heating to temperatures under 100°C., these gels can be reconstituted into pastes by adding the required water and then heating and stirring. It has not been proved, moreover, that stale bread refreshened by heating is identical physicochemically with freshly baked bread.

Finally, it is often claimed that the retrogradation of starch in a paste proceeds more slowly than the staling of bread. However, considering the great differences in experimental conditions for the starch when prepared simply as a paste and in a bread mixture, this argument does not seem important.

On the other hand, strong evidence that retrogradation in starch is concerned with staling follows from the observation of Katz that acetaldehyde and propionaldehyde will prevent or greatly retard staling. Although the action of aldehydes on starch is admittedly complex, it seems quite certain that in the early stages of this action a loose bond is formed with the carbohydrate through hydroxyl groups. The starch becomes more difficult to hydrate and requires substantially higher temperatures and higher concentrations of alkali to disperse or dissolve it. If untreated starch pastes are allowed to age, there is a gradual reduction in the intensity of the blue color with iodine as the retrogradation process is in progress; that is, the starch molecules gradually orient with themselves and become less able to orient with iodine molecules. The presence of a small amount of acetaldehyde in the starch paste, however, will prevent the loss in the ability of the paste to form a blue color with the iodine. Also, if the acetaldehyde is removed, the normal decrease in intensity of the blue iodine coloration begins at once. Similarly, bread may be kept fresh by added acetaldehyde and if the acetaldehyde is removed, by passage of a current of moist air through the bread, the staling process starts at once and follows its normal course.

The above facts are explained on the assumption that the bonds of association of the starch molecules with aldehyde and with each other are of the same type and through similar groups in the starch molecules, which groups are very likely the hydroxyl groups. The association of the starch molecules with iodine, however, is of a different type, as discussed elsewhere in this volume.* The latter is essentially a sorption effect in which the starch chains coil around the iodine molecules. Hence, whereas acetaldehyde can prevent hydroxyl association by a blocking effect, it does not prevent the chains from forming coils. Removal of the aldehyde, however, allows cross-bonding and obviously the formation of coils by each separate chain becomes more difficult. It is logical to suppose that the aldehyde tends to act on the starch in bread in a similar fashion, and in consequence orientation by cross-bonding is made more difficult and staling is prevented until the blocking influence is removed.

In conclusion, although staling may not be due exclusively to retrogradation, as the term is used in the more usual sense to denote the less reversible orientation of the linear molecules, it can be concluded that a related change in the starch, hydrogen bonding of hydroxyl groups, both on linear and branched configurations, with the probable elimination of one or more water molecules per bond formed,† is the principal action in staling. Quite naturally, as suggested by Alsberg and others, the change in the physical state of the gluten as the bread cools and ages cannot be entirely eliminated from an explanation of the total effect observed. Since this view was first given by the writer (36), two groups of workers have presented data to show that a modification within the amylopectin fraction of starch is involved in the staling process.

---

*See Chapter XVII.

†See structural formulae I and II, Chapter IX.

Noznick *et al.* (37) found that bread made from synthetic flours containing wheat gluten and waxy maize starch became stale at at least the same rate as bread made from wheat gluten and wheat starch. Schoch and French (38) found in measuring the progressive decrease in cold water soluble extract of bread crumb as it stales, that, first of all, the extract of fresh crumb was largely amylopectin (low iodine affinity) and secondly, the amount of water-soluble amylopectin within the crumb decreased as staling progressed. Indeed, both groups of workers, particularly Schoch and French, are inclined to attribute staling entirely to a spontaneous aggregation of the amylopectin fraction.

On the other hand, during this same interval, an antistaling agent for bread, polyoxyethylene stearate, has been described by Favor and Johnston (39), the action of which is shown to be more specific for amylose than for amylopectin. When added to pastes containing amylose, set-back on aging was very materially reduced, whereas, when the antistaling agent was added to pastes of waxy maize starch the cold paste viscosity was not altered materially in comparison.

Much could be said on both sides of the question from the data of all three groups. Bread made from flour the starch of which was all waxy maize was too soft and gummy to permit reliable crumb softness measurements and was more fresh, based on crumb swelling tests after the aging or staling period than was fresh bread, the starch of which was all wheat. Furthermore, it is to be noted that freshly baked bread is largely insoluble (90-95%) in cold water and the staling effect is concerned with changes in the 95% insoluble fraction as well as changes in the 5% soluble. Staling is not, therefore, an insolubilization process, and this effect is significant only insofar as insolubilization of the last few per cent of the bread reflects, or is a part of, the major reaction which is an extension, both in number and kind* of the formation of associative bonds between starch molecules, both linear and branched.

3. Crackers and Biscuits.** Starches are used in the biscuit industry for two purposes: as an aid to manufacturing and as a biscuit ingredient. When powdered sugar is not used soon after grinding, it is common practice to add 3% of redried corn starch to prevent lumping. Corn starch is generally employed for dusting the canvas aprons on icing machines to prevent sticking. Starch would be an ideal agent for dusting some biscuit doughs during machining were it not for the fact that the starch "bridges" and hence

---

*As for example, hydrogen bonds forming directly between carbohydrate hydroxyl groups which at first, perhaps, are from carbohydrate hydroxyl to a water hydroxyl group and thence to another carbohydrate hydroxyl.

**Contributed by T. J. Otterbacker.

26

does not feed evenly in the mechanical spreading and shaking devices. Recent advances in producing a free-running starch should eliminate this difficulty.

Perhaps the oldest use of starches as a biscuit and cracker ingredient is that of arrowroot starch or "flour" in the widely produced arrowoot cracker or "hard-sweet." Arrowroot biscuits, because of their starch content, have been regarded as the most suitable of all biscuits for children, infants, and invalids, and arrowroot starch was for long considered to have almost a medicinal value. The reasons for this high esteem, in the opinion of the author, are (*a*) it was one of the first relatively pure starches of commerce to be produced; (*b*) the price of the starch always has been comparatively high; (*c*) it originates from the semi-tropics; and (*d*) early publications (40) indicated that arrowroot starch is the most easily saccharified of all starches by the saliva.

The arrowroot cracker was and still is a most nutritious and easily digested baked product as the following old standard formula indicates. However, inspection of the table of ingredients shows that these qualities can hardly be due solely to the presence of the arrowroot starch.

English Arrowroot, Hard-Sweet

200 lbs. soft flour*
25 lbs. arrowroot flour
20 lbs. invert sirup
45 lbs. butter
65 lbs. powdered sugar
20 lbs. eggs
2 lbs. salt
4 lbs. cream of tartar
2 lbs. soda
vanilla to suit
20 lbs. whole sweet milk (variable)

Method—Cream arrowroot, shortening, sugar, eggs; add vanilla and salt. Sift soda in flour and add. Put sirup on top of flour with milk and make semisoft, smooth, plastic dough. Break down and let stand 2 hours. Rebreak and roll well. Run medium thin. Bake on wires.

27

The chief ingredient of most biscuits and crackers is wheat flour of which about 80% is wheat starch. Yet no attention has been paid directly to the role of wheat starch in the industry. The wheat starch becomes important by difference, so to speak, because it is the principal ingredient the flour contains in addition to protein. Biscuit flours are "soft" flours and have a protein content, roughly, of 7.0 to 8.5% for sweet goods and cracker doughs and of 8.4 to 10% for cracker sponges.

In order to give desirable eating qualities to the baked products as well as to avoid the use of an exceptionally high proportion of sugar and shortening which would otherwise be required, it is necessary to keep the protein of the flour sufficiently diluted either with the natural wheat starch or with some added starch such as corn or tapioca. Flours which contain less than 7.0% of protein (approximately, but the amount varies with the flour) exhibit most of the characteristics of a starch paste when they are used for baking, while those which contain a higher protein content exhibit more of the characteristics of a "dough."

---

*In this and the following formulae, flour denotes wheat flour unless specified to the contrary.

It is desirable for production of sweet goods to keep the flour as near to this critical composition as possible. Because of the differences in the annual crop, prices, etc., such flour is often not available. It becomes necessary then either to increase the sugar and shortening content of the biscuit or to dilute the protein with some other ingredient. Corn starch is usually selected for this latter purpose. During World War I, good biscuits were made with flour diluted with as much as 50% of corn starch. This, however, is more than the optimum dilution. By using 5 to 20% of starch (based on flour weight), depending on the protein content of the flour, a 15% saving in the amount of sugar and shortening results. In "honey" doughs it is possible to eliminate the shortening entirely. In a soft batter dough, such as the vanilla wafer, a 12.5% reduction in the amount of sugar and shortening can be effected. Furthermore, the wafer dough does not tend to stiffen and to become tough during the time after it leaves the mixer until it is through machining. The baked product is also more tender than biscuits which are made without starch.

Both raw and gelatinized corn starch are used in the manufacture of crackers. When the flour is of normal strength, the addition of 10% of corn starch will save an equivalent amount of fat and 15% of the yeast and at the same time the starch addition produces a more tender cracker. When the flour is very "strong," "bucky," and generally hard to handle, the addition of about 5 to 10% of a gelatinized corn starch product* with a cold water absorption of about 10:1 will enhance fermentation, give a smooth running dough, and result in the production of a cracker which does not buckle or "cup" during the baking processes.

The advantages of using corn starch for baking biscuits and crackers may be summarized as follows: (*a*) the flavor is improved; (*b*) greater effectiveness of the added flavors is secured; (*c*) the interior color of the biscuit is clearer; (*d*) there is a saving in the amount of shortening required; (*e*) saving of sugars; (*f*) saving of yeast (crackers); (*g*) there is a reduction of sticking of finished goods (this is especially true of "honey" goods in warm weather); (*h*) the grain or texture of the baked product is improved; (*i*) a much better bottom face is produced on the cookies. 2.6

---

*Examples of the type of product indicated are those such as are sold under the trade names "Amijel" and "Amidex."

In bake shop specialties, such as the ice cream cone or the sugar wafer shell, the character of the flour is even more important than in regular sweet goods and crackers. A flour that is too "soft" will make a product which will crumble easily, while a flour which is too "strong" will produce a brittle shell. In this case, also, the proper flour is rarely available or the cost makes its use prohibitive. Hence, both corn and tapioca starch are used by the industry to modify the characteristics of the flour. Tapioca has been preferred to corn starch because tapioca appears to give a more tender cone or shell which is less brittle, and consequently less breakage of the product in packaging and shipping results. A better flavored shell may be made when tapioca starch is used. Lastly (probably the most important reason of all), the price of tapioca in the past has been lower than that of corn starch. As high as 40 to 50% of starch (based on flour) may be used to make these bake shop specialties. The average amount of starch used, however, is probably 5 to 7%, and this value rarely exceeds 15%.

Occasionally potato starch and flour are used to produce a specialty product, although their use is not common in the biscuit and cracker industry. The accompanying formula is an illustrative example of a potato cracker.

Potato Cracker

100 lb.	flour	2 lb.	salt
40 lb.	potato flour	5 lb.	cheese
20 lb.	potato starch	16 lb.	butter
40 lb.	boiled potatoes	20 lb.	water

Directions—Put potato flour, starch, and boiled potatoes in the mixer; mix for 5 minutes. Add water, salt, cheese, and butter; mix for 5 minutes. Add flour and mix clear. Top with fine salt. Spray with coconut oil after baking.

Because of the low moisture content (4 to 6%) of finished biscuits and crackers, the retrogradation of starch is not a factor in their staling, except when they are allowed to absorb abnormal amounts of water. The keeping quality of the shortening is usually the limiting factor.

The accompanying general skeleton formulae illustrate how corn starch is used in standard biscuit and cracker products. For the purpose of simplicity all formulae are based upon 100 parts of flour. In practice, the size of the batch of dough varies with the capacity of the essential equipment for handling it. The ability of the dough to withstand aging is also a factor which limits the size of the batch.

Soda Crackers (Peeling Machine)

Sponge:

80 lb. flour
¼ lb. yeast
½ lb. dextrose
5 lb. gelatinized starch
32 lb. water

Preferred Mixing Order and Method—Yeast, water, yeast foods, part flour, starch; ferment. Set at 72°F., out at 82°F. after 18 hours.

Dough:

20 lb. flour
12½ lb. shortening
1 lb. salt
2 lb. water
½ lb. soda

Preferred Mixing Order and Method—Add all ingredients; ferment. Out at 84°F. after 5 hours.

Graham Crackers (Cutting Machine)

75 lb. flour
25 lb. graham flour
10 lb. starch
12 lb. shortening
13 lb. sugar
13 lb. dextrose
5 lb. invert sirup
1 lb. salt
1 lb. soda
¼ lb. ammonium bicarbonate
22 lb. water

Preferred Method—Make a hot sirup (200°F.) of all sugars, salt, and water, and add to all other ingredients in the mixer. Bring dough out at 125°F. Run before dough cools off.

Honey Type (Cutting Machine)

100 lb. flour
20 lb. starch
75 lb. sirup (invert, honey, dextrose, and molasses) 80% solution
0–6 lb. shortening
2 lb. soda
14 lb. water

Preferred Method—Mix all ingredients except flour and starch. Add starch and flour.

Semihard Sweet (Cutting Machine)

100 lb. flour
5–10 lb. starch
28 lb. sugar
12 lb. dextrose
1 lb.salt
16 lb. water
3 lb. eggs
½ lb. soda
½ lb. cream of tartar
0–5 lb. shortening

Preferred Method—Cream sugar and shortening; add eggs; add water; add flour and starch.

Fig Bar Dough (Special Machine)

100 lb. flour
5 lb. starch
30 lb. sugar
12½ lb. dextrose
2½ lb. invert sirup
20 lb. shortening
8 lb. eggs
3 lb. dry skim milk
20 lb. water
½ lb. soda
¼ lb. cream of tartar
1 lb. salt

Preferred Method—Cream sugar and shortening; add sirup; add eggs; add water; add starch and flour.

Honey Dough (*Wire Cut Machine*)

100 lb. flour
10–20 lb. starch
70 lb. sirup (honey, invert, dextrose, molasses)
15 lb. sugar
0–5 lb. shortening
1 lb. salt
1 lb. soda
½ lb. ammonium bicarbonate
½ lb. cream of tartar
22 lb. water

Preferred Method—Mix flour, starch, sugar, and other ingredients. Thin down with water slowly.

Wafer Dough (*Wire Cut Machine*)

100 lb. flour
20 lb. starch
50 lb. sugar
17 lb. dextrose
33 lb. shortening
5 lb. dry skim milk
10 lb. eggs
1 lb. soda
½ lb. cream of tartar
½ lb. ammonium bicarbonate
1 lb. salt
65 lb. water

Preferred Method—Cream sugar and shortening. Add eggs; add water; add starch and flour.

Scotch Shortbread (*Rotary Machine*)

100 lb. flour
10 lb. starch
20 lb. sugar
15 lb. dextrose
10 lb. eggs
10 lb. water
½ lb. soda
¼ lb. cream of tartar
35 lb. butter and (or) shortening

Preferred Method—All ingredients in mixer at once give good results.

Sugar Wafer Shell (*Special Machine*)

(Cones are similar.)

100 lb. flour
7 lb. starch
2 lb. shortening
75 lb. water
75 lb. milk
½ lb. soda
¾ lb. salt
¼ lb. ammonium bicarbonate
1½ lb. egg yolk

Preferred Method—Mix all ingredients together at once except the water and milk. Thin down gradually with these liquids.

Tapioca and corn starches were once considered important ingredients for use in the biscuit marshmallow process. The following skeleton formula is typical.

21 lb. water
31 lb. corn sirup
44 lb. sugar (partially inverted)
1.6 lb. *Cassava* starch
2.4 lb. gelatin

Preferred Method—Suspend the starch in part of the water. Add the sugars. Heat to 200°F.; add the corn sirup. Add gelatin which has been dissolved in part of the water. Cool to room temperature and beat in convenient batches.

As the art of compounding biscuit marshmallow progressed, the starch was withdrawn from the formula. The consensus of opinion among biscuit bakers has been that the starch has a tendency to hold down the volume of the marshmallow. It does, however, contribute a certain degree of "set," or stability, to the marshmallow, which characteristic is particularly useful in summer weather.

Lately, the practice of adding 0.25 to 0.50% of a powdered, gelatinized corn starch product (cold water absorption about 10:1) at the beater has received favorable attention. The added starch product permits the marshmallow to carry 1 to 2% more water, which promotes tenderness. At the same time the starch gel increases the stability of the marshmallow for machining, packaging, and shipping.

Preferred Method and Typical Seasonal Formulae for Biscuit Marshmallow

Stock sirup	Winter	Spring and fall	Summer
	lb.	lb.	lb.
Sugar	5.0	10.0	20.0
Dextrose	5.0	10.0	13.0
Corn sirup	25.0	27.0	30.0
Invert sirup	55.0	36.0	17.5
Water	8.2	14.8	17.0
Gelatin (175 bloom)	1.5	1.75	2.0
Gelatinized starch	0.3	0.40	0.5

Method—Make a sirup of the sugars, sirups, and part of the water. Dissolve the gelatin in part of the water. Add the gelatinized starch and the gelatin solution at the beater.

Corn or tapioca starch is sometimes used to break up the excessively hard, crystalline structure of flat and "trolley" icings. These icings form a thin, semitransparent coating of small sugar crystals which are held together by a small amount of sirup. Illustrative formulae are given.

Flat Icing

26.5 lb. water
47.0 lb. sugar
5.6 lb. corn sirup
0.9 lb. gelatin
5.0 lb. powdered starch
15.0 lb. powdered sugar

Method—Dissolve the sugar and corn sirup in part of the water at 200° F. Add the gelatin dissolved in part of the water. Beat to 150° F.; add the powdered sugar and starch, and then beat to the proper consistency.

Trolley Icing

20 lb. water
2 lb. corn sirup
72 lb. sugar
6 lb. starch, powdered

Method—Mix the ingredients and keep at 105–110° F., stirring continuously

4. Salad Dressing. During recent years an extensive market has been opened to starch with the development of new formulations for salad dressings. The most important of these are of the mayonnaise type. Mayonnaise consists essentially of a vegetable oil emulsified with egg albumin, vinegar, mustard, salt, and other less important condiments. The newer salad dressings differ essentially in the fact that the vegetable oil is emulsified with a relatively large proportion of gelatinized starch and less or, in the extreme case, no egg albumin. The result is also that the percentage of oil in the dressing is appreciably reduced. The newer type of salad dressing may therefore be made with considerably less cost, since the price of both vegetable oil and eggs is decidedly more than that of starch.

The newer dressing is preferred for dietary reasons in some cases because of this alteration in composition. No intimation is intended that the nutritional value of starch is superior to that of proteins and fats. All three types of foods have a very proper place in a well balanced diet. Carbohydrates furnish energy and body warmth as do fats. The latter, however, after saponification, may be more readily stored as fat tissue, if there is no immediate need for energy. The utilization of fats for energy depends upon carbohydrate utilization. In simple words, fats may be said to burn in the flame of the carbohydrates. The presence of substantial quantities of available carbohydrates, therefore, facilitates the utilization of fats, especially when large quantities of the latter are eaten, as for example in a rich salad dressing. Proteins, on the other hand, are a source of building material for muscle and other tissues. This is to replace normal wear and tear in adults and to facilitate growth in children. An overabundance of protein, so that the system is forced to call on protein for energy requirements to a large extent, places an undue strain on the deamination and other nitrogen-converting functions of the liver and overtaxes the kidneys to eliminate excesses of these nitrogenous bodies. The normal adult, particularly one who is inclined to obesity, might find the newer salad dressings less conducive to an increase in body weight than the former type. The active person will secure a more balanced composition to utilize for energy requirements.

The use of starch in this field depends upon its emulsifying power, as stated above. Starches are, however rather poor emulsifying agents; that is, the emulsifying power of a gram of starch is much less than that of a gram of a standard emulsifying agent, such as soap. Stable emulsions may be made with gelatinized starch, but they require a relatively large proportion of starch and special methods to accomplish the result. The use of starch to emulsify vegetable oils has been reviewed recently by Goikhman (41, 42).

The use of a relatively large proportion of starch to produce a stable emulsion with oil in salad dressings requires that another characteristic be imposed on the starch intended for this use. The gelatinized starch added must not change the texture or other characteristics of the salad dressing which the consumer has become accustomed to accept as standard. Some starches tend to gel; some tend to develop a "short" consistency. Furthermore, some starches will resist the tendency to set a firm gel because of the oil present. On the other hand, in such a medium they will show a pronounced tendency to retain less water. A syneresis develops, and the salad dressing begins to separate a water phase.

The gelatinized starch must not be adversely affected by the action of other ingredients present. Salad dressings are normally quite acid because of the added vinegar or acetic acid. Some starch pastes are very materially thinned in the presence of as weak an acid as acetic. In this case, the decrease in viscosity also is paralleled by a decrease in the emulsifying power. The result is first an undesirably thin salad dressing and later an unstable one. Some starches are more susceptible to a physical breakdown when cooked with sugar than are others, and many formulae for the newer salad dressings contain substantial amounts of sugar.

Very few starches indeed meet all the requirements already listed plus those imposed by the manufacturing operation to be described. Normally, therefore, a combination of starches is selected, two or more types being used to impart the desired characteristics to the final gelatinized mixture. Corn starch pastes have more body when cooled to room temperature than the more common non-cereal starches, but they are inclined to be of "short" consistency or, if unmodified, to gel. Corn starches are, however, fairly stable in the presence of acid such as acetic and, once the gel structure has been broken down, the remaining body is fairly stable in the remainder of the emulsifying operation. When they are unmodified, their water retention is not as good as that of some of the other types of starch. Tapioca and some of the other non-cereal starch pastes are softer and are fairly good emulsifying agents but, by themselves, produce a body which is too fluid or mobile. They are weaker in the presence of acetic acid and when cooked with sugar. A common practice is to use a combination of some corn starch and some tapioca.

Nearly each manufacturer has formed his own idea of the proportions of each starch to use, and these formulations show a wide variation. Surprisingly enough, however, the end-product in each case varies little in major characteristics from those produced by use of other formulations. The explanation is a relatively simple one. During the development of the newer salad dressings, few modifications of either corn or tapioca starches were available for this purpose. Indeed, in many cases, the maker of salad dressing used unmodified starches of both types. He installed the type of emulsifying or homogenizing equipment which was deemed to be most suitable for his purpose and experimented with different combinations of corn and tapioca starches until the desired result was obtained.

Emulsifying equipment varies in design from that at one extreme, which is little more than a beater, to the other extreme type, which may be a high pressure homogenizer. In between is the colloid mill type of emulsifier. The proportion of corn starch or modified corn starch to tapioca which is found to produce the desired body and other characteristics in a beater type of emulsifier quite obviously would not be expected to produce the same characteristics in a colloid mill type. Nor would a proportion of a given modified corn starch be expected to be interchangeable with a like proportion of another modification, when the same type of equipment is used. Formulae must therefore be varied to suit the type of equipment installed by each manufacturer of salad dressing.

The attempt of the starch manufacturer to introduce one particular modified starch, *e.g.*, a corn starch, to meet all requirements met with some difficulty. The result is that several types are marketed to suit varied conditions. Attempts to use these types interchangeably has frequently led to disappointing results for the maker of salad dressing.

Formulations will be found to vary for another reason. Salad dressings are of two general types which may be divided on the basis of oil content, a salad dressing of high oil or of low oil content. Some manufacturers market both types. With most equipment used, the ratio of the heavier bodied corn starch is increased in salad dressing of the high oil type.

An example of the manufacturing process, as it relates to the utilization of the starch, is as follows: The following ingredients are mixed in the proportions, by weight: corn starch, 6; tapioca, 4; acid (100 grain vinegar), 1; mustard, 1; salt, 1; sugar, 20; water, 67. The mixture is cooked in a closed, jacketed kettle for 30 min. at 180°F. The paste is dropped into a cooler, where it is reduced in temperature of 90°F. and then poured into containers for storage. About 18 to 24 hrs. at 50°F. are sufficient to complete the primary setting of the paste. After the storage period, the paste is added to the oil and egg, in the emulsifier, in the desired proportions. This paste has been used in the thick salad dressings of high oil content. The ratio of paste to oil, however, will be accurately adjusted, depending on (*a*) the ratio of egg to oil used and (*b*) the homogenizing equipment available for use.

The ratio of tapioca to unmodified corn starch in the mixtures used varies normally between 33% tapioca to 67% corn starch and 67% tapioca to 33% corn starch.

Present research is concerned with the production of a single starch which will avoid the necessity of using mixtures. Several grades will most likely be required to meet the varied manufacturing processes, as stated above.

5. Starch in Baking Powder. Baking powder consists essentially of an acid or acidic substance and a carbonate, both in dry form, which, in the presence of water, react to liberate carbon dioxide gas. When this gas is liberated within a dough, leavening results as the temperature is raised in the baking operation, owing to the thermal expansion of many minute gas pockets which have been trapped by the glutenous portion of the flour. The acidic component, for example calcium acid phosphate, sodium acid tartrate, or sodium aluminum sulfate, is usually such a feeble acid that heat is required to promote its reaction with the carbonate (*e.g.*, sodium bicarbonate). The reaction is appreciable at lower temperatures, however, and indeed some manufacturers claim that their product begins to react as soon as it is stirred into the batter. To maintain the efficiency of a prepared baking powder in storage, therefore, the two opposing ingredients, the acid and the carbonate, should be kept dry and kept from too intimate contact in spite of the fact that both are mixed together as fine powders. Accordingly, an inert material is added which acts as a diluent. The latter should be pure and edible, preferably white, free from characteristic odors, and capable of maintaining a free-flowing, powdery condition under adverse circumstances. Corn starch admirably meets all of these requirements, especially if processed for this use. The manufacture of baking powder represents not only one of the largest outlets for corn starch prepared for food purposes, but also it is one of the largest of applications for which the starch is not utilized for its paste or sol forming property.

In a related use, the blending of starch with confectioners' sugar, an advantage in the use of starch is that when it is dried to a low moisture content it may act as a moisture "acceptor." That is, when dehydrated to the range of 5% moisture, the starch will, owing to its hygroscopicity, preferentially absorb and adsorb about 7% or more of its weight of moisture, should the mixture be exposed to a high relative humidity or dampness. At 12% moisture content, the starch still retains a relatively free-flowing condition, and i specially processed, starches result which are extremely free-flowing even a 14 to 15% moisture content. The moisture-binding power of starch is o additional value in baking powder in that it tends to keep the acid and carbonate in a drier state and hence tends to prevent them from losing their leavening efficiency through premature reaction. However, when native starch i dehydrated by redrying, it frequently develops a characteristic odor i storage. In the case of corn starch this is due to the chemical instability o certain non-carbohydrate substances associated with the starch, which condition is promoted by the redrying operation. This characteristic property of starch may be altered by a treatment of the starch before dehydration. Chapter III has dealt with the control of starch odors as well as methods to obtain a white and mobile, powdered starch.

By dry starch "mobility" is meant the freedom to flow, particularly through sieves or sifting devices. The rate at which a fixed weight of starch will pass a given mesh sieve, when a limited amount of agitation is used, may be taken as a relative measure of mobility, as described in Chapter VI. This obviously desirable characteristic of a good baking powder starch parallels another property important for this use. Kerr* has observed that the mobility of dry starch is directly proportional to its apparent volume per unit weight; the more freeflowing the starch, the greater the volume occupied by a pound of the starch. In addition to its appeal to consumers who prefer to buy on a volume basis rather than weight, that is, prefer to receive a larger package for a certain price, the property of contributing a greater volume for a definite addition of starch by weight possesses a real merit as well. A greater volume per unit weight in a product containing the powdered starch indicates that there is more free space in this mixture, which in turn allows the conclusion that the ingredients separated by the starch are in less intimate contact. This condition is obviously desirable in respect to baking powder.

One of the most practical ways to secure high mobility in a dry starch is to treat the starch when wet with oxidizing agents under controlled conditions, as indicated by Kerr.† This worker has also patented a process in which oxidizing agents are also used for producing a white starch much less disposed to develop undesirable odors when dehydrated and stored (43).

6. Confections. In addition to the wide-spread use of derived products from starch such as dextrose and confectioners' corn sirup as sweetening agents in confections, starch and modified starches are also used in the manufacture of several types of candies. Dextrose and corn sirup are employed in the manufacture of hard candies, fudge, nougats, marshmallow, gums, fondants, and coatings as well as other types of confections. Starch is used principally in the manufacture of gums, pastes, and panned sweets, either as an ingredient of the confection or as an aid in manufacture as for the production of molds. The technology of candy manufacture has been given by Jordan (44), Scarborough (45), and others.

---

*Unpublished data.

†See Chapter III.

A variety of gum confections are produced by the use of starch. The nature of the product depends not only on the formulation used but also on the cooking and finishing procedures. The formulation may be varied by the addition of colloids other than starch, such as gelatin and gum arabic, or by the choice of the starch employed. The most popular gum in America is made from a starch jelly to which no other colloid is added, and when a thin boiling starch such as corn starch is used the end-product may be a gum drop, gum slice, or jelly bean. It is possible to boil out a relatively large amount of water from a formulation which contains starch, corn sirup, and sugar, leaving the starch jelly in a stiff and gummy condition. When this gum is cast and allowed to dry, the chewy type of confection results. By the use of a shorter cooking time (or by cooking to a lower final temperature) more water is left in the starch jelly, and softer confections with a "shorter" texture are obtained. The gelling power of starch is preserved and after being poured into molds to set to shape, the gum may have a moisture content of about 20%. These are the familiar gum drops or gum slices.

Gum drops are commonly made by the use of thin boiling corn starch for three principal reasons. Corn starch has excellent gelling properties and therefore is preferred to the softer setting starches such as tapioca. Sago starch has good gelling properties and has been used for this purpose. However, gums made from sago starch have a tendency to become more opaque and dull after aging than gums from corn starches which are prepared for this use. As a starch is modified by acid treatment to produce the usual thin boiling variety, the hot paste viscosity of the starch is reduced, but modifications, *e.g.*, fluidity grades of 40 and 60, produce pastes which show a more pronounced tendency to gel (when considered in relation to their hot paste viscosity) than an unmodified starch. Therefore, by the use of a slightly greater amount of thin boiling starch, a hot paste viscosity results which is equivalent to that of the unmodified starch, but when the two hot pastes of equivalent viscosity are cooled, the gel strength of the thin boiling starch paste is considerably greater than the gel strength of the paste of the unmodified starch. Thus, by the use of a thin boiling corn starch it is possible to adjust the formulation so as to prepare a cooked mixture that possesses a high degree of fluidity when hot and a satisfactory gel structure when it is cast and allowed to set, even though the gel contains 20% or more moisture. A fluid hot paste is desirable, since it facilitates the pouring of the cooked starch jelly into molds. The manner in which the starch is modified to reduce its hot paste viscosity is chosen so that a "stringy" characteristic does not develop in the hot starch jelly, for if it does the flow of the gum into the molds will not break abruptly after the mold is filled and the kettle is moved to the next mold. This results in castings which are connected by thick threads of the gum, which represent a loss in the yield of finished goods.

The relative increase in gelling power which results when corn starch is acid-modified is a function of several variables employed in this conversion. Normally these modifications are carried out by converting the starch in a suspension with 0.1 N H_2SO_4 at 125°F. for from 10 to 15 hours. As the concentration of the acid employed is reduced, that is as the pH is decreased, and as the time of the conversion is increased, thin boiling starches are produced (*e.g.*, starches with a fluidity of 60) which cook to measureably softer setting pastes. Conversely, as the acidity is increased and as the conversion time is reduced, a thin boiling starch is produced with more pronounced gel strength. A relatively high concentration of acid may be used to make corn starches of 40 or 60 fluidity by the use of converting temperatures lower than 125°F. An illustration in which the above effect is employed to advantage in the manufacture of a gum drop starch is the process patented by Meisel (46).

Clarity of the gum is a prime requisite for some types of confections. Although both corn and sago starches form pastes of high gel strength, both give gels which cloud in time after they are cast. Relatively clear gums can be made from corn starch after it is acid-converted to a fluidity higher than 60. However, the gel strength, per gram of the cooked starch, is also reduced and soft gums result. Oxidation of corn starch, *e.g.* by a treatment with sodium or calcium hypochlorite, or by sodium peroxide, modifies the starch so that it cooks to a very clear paste, but the gel strength is very low. On the other hand, Kerr (47) has observed that corn starch may be oxidized with calcium peroxide under special conditions to produce a starch of high potential gel strength and one which makes an exceptionally clear gum drop.

The gums are shaped after the cooking procedure by pouring the hot jelly into molds. These are made by impressing the desired shapes in a layer of starch. A special corn starch is suited for this purpose. It is usually dried to a low moisture content to increase its water-absorbing capacity, and a small amount of an edible oil is thoroughly blended with the starch so that it will retain the shape of the impressions made in it. The trays of starch into which the gums are poured are placed in a hot room to adjust the gums to the correct moisture content and to set the gel. The molded gums are then shaken loose from the starch and are freed from any remaining starch by brushing. The starch is reconditioned by screening and by an occasional redrying treatment. Fresh molding starch is added to compensate for losses.

After the gums are brushed they may be finished by rolling in sugar crystals to apply a sweet crystalline surface to the gums, or for a similar result they may be wet with a saturated sugar solution which subsequently crystallizes on the surface of the gums. The more extensively cooked gums, such as jelly beans, may be finished by a panning operation. The confections are placed in a rotating kettle where by a tumbling action a sugar coating is built up by adding a sugar sirup progressively in small amounts. After the coating has been built up to the desired extent the operation is continued so that the exteriors develop a gloss. Frequently ingredients are added in the panning operation which act as polishing agents.

7. **Additional Uses.** Starch sold to the brewing trade for use in the manufacture of beer is usually in the form of grits, that is, a partially gelatinized or otherwise treated starch which is more readily susceptible to the malt conversion operation of the brewer to hydrolyze it to fermentable carbohydrate than untreated starch. The entire converting reaction, including the primary mashing procedure is principally a saccharification. Production of dextrins is thought to increase the difficulties encountered by the brewer in the production of a clear beer. It is to be noted, however, that European beers, many of which are made with no added starch, are frequently stored and served at room temperatures since lower temperatures materially increase their haziness. Nevertheless, starch producers take particular pains and use special processes to produce brewers' grits which malt readily. The usual gelatinized starch product such as is made in roll-drying operations and in which retrogradation and associative reactions may have ensued during or after manufacture is, of course, less suitable than one in which granule structure within the grits has been weakened by other means. Corn starch is largely used for this purpose although some brewers prefer rice.

Starch puddings are of three general types, the pearl tapioca type consisting mainly of sizable aggregates of starch granules in the form of "seeds," the *blanc mange*, or smooth corn starch pudding type, similar to a custard and the so-called Danish dessert type which resembles a gelled fruit juice, or gelatin dessert. Apparently, the tapioca type may be duplicated by the use of corn and waxy maize starches in a manufacturing process recently patented by Schopmeyer (48). This directs the making of a dough of about 10 parts of corn starch to about 90 parts of waxy maize at a moisture content of 40-42%. The dough is heated with stirring to about 160°F., during which operation the mass becomes semigelatinous. Stirring is continued until coherent pasted pellets form. The corn starch promotes setting of the pellets to a firm condition, prevents the gelatinized pellets from becoming too fluid when they are subsequently cooked in a pudding and promotes setting of the pudding on cooling. The behavior of waxy maize starch alone is unsatisfactory, at least in this process.

The typical corn starch pudding of former times which was cast into a rigid form in molds has been modified in recent years so as to form a softer, less coherent, custard-like texture. This was accomplished at first by the use of a blend of starches such as corn and arrowroot. Latterly, the corn starch has been modified in manufacture so as to produce the same, or better result by itself.

Originally, the Danish dessert type, or Danish *rödgröd* was made from unmodified, or native starches such as potato, and although relatively clear, possessed a long body and somewhat mobile consistency. More recently, products have been made which retain the clarity of the tuber starch pastes and in addition, set to gels. This has been accomplished by treating non-cereal starches with agents to create relatively stable cross bonds between the carbohydrate molecules such as, for example, by procedures outlined by Kerr and Schink (49) in a recent patent. On the other hand several patents have been allowed in recent years for modifying potato starch so as to form a corn starch type pudding. Usually, the processes consist of a mild hydrolytic treatment, or limited oxidation such as in the patent granted to van der Meer (50).

In addition, a new type of pudding mixture has lately appeared on the market which requires only mixing with cold water or other fluids in order to create a pudding which resembles the *blanc mange* type of dessert in general characteristics. A patent application (51) describes the production of a cold water soluble pudding powder by oxidation of potato starch.

In the baking of pies and related products, the type of starch used is determined by the desired character of the filling. For example, these may be of the cream or berry type of filling. For the production of pies with cream fillings, such as Boston cream, coconut cream or lemon cream, a considerable amount of modified corn starch is used of the grade employed in puddings. One obstacle encountered when a prepared mixture is sold for lemon fillings is that the acidic ingredients, the fruit acid, hydrolyzes the starch on storage so that a thin filling may result. To overcome this some manufacturers have separated the fruit acid from the other ingredients by a gelatin capsule, or other coating which readily dissolves and releases the fruit acid when the filling is mixed for baking. Some pie bakers starches may contain a very small amount of sodium bicarbonate to buffer the fruit acid in cooking.

In the case of berry fillings, such as cherries and blueberries, the starch is added to give body to the filling so that the juices will not soak into the crust, or run, when the pie is cut. Furthermore, the consistency should remain very nearly the same whether the pie is cut within a few hours after baking or after standing for a day on the baker's shelves. Several starches fulfill these requirements. Untreated corn and cereal starches tend to set up too firmly with age, producing a pudding-like consistency, they become opaque and tend to mask the natural color of the fruit. Tapioca and other unmodified root starches give a body which is undesirably fluid and if used in excess to create more body may give a gummy texture. Accordingly, starches are treated in manufacture so that they will add to the body of the berry filling without unduly changing the natural appearance of the juices. Rather excellent starch products have been made recently. Root starches, cross-bonded by procedures given by Kerr and Schink (49) and a waxy starch product described by Deutsch (52), presumably also stabilized by a cross-linking reaction, are two notable examples.

A rather large amount of starch is used by the canned food industry such as in the production of creamed soups, baked beans and some other varieties of canned vegetables. One of the major problems in this industry is to can the food product in a sterile condition, particularly in respect to the spores of thermophyllic bacteria which resist the heat treatment in the cooking operation. Accordingly, food processors buy ingredients such as starch and sugar under rigid specifications for their sterility. This lessens the load on the food processor of eliminating, by washing and pressure cooking, the various thermophilic bacteria which are always associated with fruits and vegetables as they are harvested and brought in from the fields.

Starch is readily sterilized by a mild treatment with oxidizing agents or by heating in the dry state and several patents have been issued in this connection, which have been discussed under modified starches. However, these treatments in general have a tendency to produce a thinner starch or to make the starch more susceptible to a thinning action when added to other ingredients and cooked at relatively high temperatures under pressure. In the cooking some additional acidic materials are created and the excessive acidity exaggerates the thinning action on the starch. Furthermore, creamed soups, for example, which are not normally acidic, such as, mushroom, may be changed in texture to a curdled condition. A patent to Kerr (53) describes the use of a sterilizing agent under conditions for minimum oxidation of the starch, which, in fact increases its paste viscosity, and describes the use of an edible phosphate buffer to maintain an appropriate pH for the particular food product to which it is added, during the pressure cooking operation.

The meat processing and packing industry consumes a very substantial quantity of starches, flours and cereal products, probably of the order of thirty-five to forty million pounds yearly in America, alone. Starch products have been used in sausage manufacture since time immemorial. The use of starch in meat products has expanded in America due to the preference for the more tender type of "cold cuts" as contrasted to the hard, Continental types such as salami, and to the production of meat loaves and other specialty items on a commercial scale. The amount of starch products used in processed meat varies over a wide range from zero, as prescribed by law for sausage manufacture in at least one state, to over 90%. "Philadelphia Scrapple," for example, consists primarily of a cereal starch product mixed only with prepared meat juices.

The application of starch products, frequently called cereal binders, in the meat packing industry has been discussed in some detail by Glabe (54). The type of starch, flour or cereal product used depends first of all on the variety of meat product and the manufacturing procedure peculiar to this variety. The purpose of the binder is very nearly the same in all cases, however. This is, to maintain a firm but tender body and to keep together the three principal constituents, meat tissue, juices, or water, and fat, during the several stages of manufacture, storage and cooking. Therefore a suitable binder has good primary water absorption when the meat is chopped, frequently with added ice. The binder should hold this water during the stuffing and processing operations. If the binder does not hold its moisture during the cooking applied either during or after processing, the meat and the fat will appear to separate and this will result in a coarse-grained, unappetizing product. Preferably also, the binder should assist in keeping the fat dispersed so that it does not collect in pockets, under conditions when it tends to become liquid.

Potato starch is considered very suitable for bologna and frankfurters. Although its primary water absorption is not of a very high order, the gelatinization temperature of the starch is sufficiently low that it is enabled to bind water very effectively during and after processing operations which reach a temperature of 165°F. The starch creates a fairly homogeneous texture with the fat and meat and a firm body which permits thin slicing. Durum wheat flour, particularly the by-product of semolina milling for the macaroni trade, is used in some volume in sausage manufacture. It has fair primary water absorption and good water retaining property together with satisfactory fat stabilization qualities.

Cereal starches and flours are used as binders in meat loaves where the final processing temperatures in the baking operation are well over the gelatinization range of these starches. Recently, pregelatinized products have been used as binders because of their excellent primary water absorption. Roll-dried starches, however, are not as suitable as starch-protein mixtures, or flours, in retaining moisture and preventing fat separation during cooking.

In commercial operations, considerable starch is used in preparing special sauces. In domestic cooking, however, sauces and gravies are normally made from flour which in many respects is superior to unmodified starch, and principally because of the apparent reluctance of the starch manufacturers to supply a packaged item of a special starch suitable for these several home uses. Starch manufacturers have, however, given considerable attention recently, together with flour millers, to the preparation of small packaged items containing prepared mixes for cookies, muffins and many similar products.

Confectioners powdered sugar may be treated to retain its free flowing property and to prevent caking in storage by mixing it with about 3% of starch. Considering the large amount of confectioners sugar manufactured, this use represents a substantial volume of starch. The characteristics required of the starch are that it be exceptionally white, that it be powdered and of relatively low moisture content and that it should remain free of any characteristic odor after it has been heated to reduce its moisture content. These properties are discussed in further detail in a patent issued to Kerr (43) for producing a white, odorless corn starch of low moisture content.

BIBLIOGRAPHY

1. Landis, Q., and Frey, C. N., *Cereal Chem.,* **13**, 281 (1936).
2. Blish, M. J., *J. Am. Diet. Assoc.,* **14**, 123 (1938).
3. Geddes, W. F., and Eva, W. J., *Cereal Chem.,* **12**, 402 (1935).
4. Sandstedt, R. M., Blish, M. J., Mecham, D. K., and Bode, C. E., *Cereal Chem.,* **14**, 17 (1937).
5. Ford, J. S., and Guthrie, J. M., *J. Inst. Brewing,* **14**, 65 (1908).
6. Józsa, S., and Gore, H. C., *Ind. Eng. Chem.,* **24**, 95 (1932).
7. Stamberg, O. E., and Bailey, C. H., *Cereal Chem.,* **16**, 42 (1939).
8. Blish, M. J., Sandstedt, R. M., and Kneen, E., *Cereal Chem.,* **15**, 629 (1938).
9. Sandstedt, R. M., Jolitz, C. E., and Blish, M. J., *Cereal Chem.,* **16**, 780 (1939).
10. Brown, H. T., and Heron, J., *J. Chem. Soc.,* **35**, 596 (1879).
11. Maquenne, L., *Compt. rend.,* **138**, 375 (1904).
12. Collatz, F. A., *Am. Inst. Baking, Bull. 9,* **72** (1922).
13. Alsberg, C. L., in Walton, Comprehensive Survey of Starch Chemistry, New York, 92 (1928).
14. Rumsey, L. A., *Am. Inst. Baking, Bull. 8,* **84** (1922).
15. Kerr, R. W., and Schink, N. F., *Ind. Eng. Chem.,* **33**, 1418 (1941).
16. Dale, J. K., and Langlois, D. P., U. S. Patent 2,201.609 (1940).
17. Kerr, R. W., Meisel, H., and Schink, N. F., *Ind. Eng. Chem.,* **34**, 1232 (1942).
18. Balls, A. K., and Hale, W. S., *J. Assoc. Offic. Agr. Chemists,* **18**, 135 (1935).
19. Jorgensen, H., *Biochem. Z.,* **283**, 134 (1935).
20. Balls, A. K., and Hale, W. S., *Cereal Chem.,* **13**, 54 (1936).
21. Ford, W. P., and Maiden, A. M., *J. Soc. Chem. Ind.,* **57**, 278 (1938).
22. Shen, T., and Geddes, W. F., *Cereal Chem.,* **19**, 609 (1942).
23. Sandstedt, R. M., and Fortmann, K., *Cereal Chem.,* **20**, 517 (1943).
24. Stern, R., *Cereal Chem.,* **21**, 81 (1944).

25. Baker, J. C., Parker, H. K., and Mize, M. D., *Cereal Chem.,* **21**, 97 (1944).
26. Swanson, C. O., and Andrews, A. C., *Cereal Chem.,* **21**, 140 (1944).
27. Elion, E., *Baker's Digest,* **19**, 31 (1945).
28. Geddes, W. F., *Can. J. Research,* **1**, 528 (1929).
29. Kent-Jones, D. W., *Cereal Chem.,* **11**, 57 (1934).
30. Blish, M. J., *Cereal Chem.,* **11**, 70 (1934).
31. Markley, M. C., and Bailey, C. H., *Cereal Chem.,* **8**, 300 (1931).
32. Geddes, W. F., Larmour, R. K., and Mangels, C. E., *Cereal Chem.,* **11**, 66 (1934).
33. Schultz, A. S., and Landis, J. A., *Cereal Chem.,* **20**, 39 (1943).
34. Swanson, C. O., and Johnson, J. A., *Cereal Chem.,* **20**, 39 (1943).
35. Katz, J. R., in Walton, Comprehensive Survey of Starch Chemistry, New York, 100 (1928).
36. Kerr, R. W., Chemistry and Industry of Starch, First Ed., Academic Press, New York, p. 391 (1944).
37. Noznick, P. P., Merritt, P. P., and Geddes, W. F., *Cereal Chem.,* **23**, 297 (1946).
38. Schoch, T. J., and French, D., *Cereal Chem.,* **24**, 231 (1947).
39. Favor, H. H., and Johnston, N. F., *Cereal Chem.,* **24**, 346 (1947); *Baker's Digest,* **21**, 124 (1947).
40. Levberg and Georgiyesky, *Societe Chemique de Paris,* New Series V, **26**, 452 (1876).
41. Goikhman, N. C., *Farmatisiya,* No. 10, 10 (**1939**).
42. Goikhman, N. C., *Khim. Referat. Zhur.,* No. 5, 109 (**1940**).
43. Kerr, R. W., U. S. Patent 2,052,308 (1936).
44. Jordan, S., Confectionery Standards, New York (1933); Jordan, S., and Langwill, K. E., Confectionery Analysis and Composition, Manufacturing Confectioner, Chicago (1946).
45. Scarborough, N. F., Sweet Manufacture, London (1936).
46. Meisel, H., U. S. Patent 2,231,476 (1941).
47. Kerr, R. W., U. S. Patent 2,268,215 (1941).
48. Schopmeyer, H. H., U. S. Patent 2,431,512 (1947).
49. Kerr, R. W., and Schink, N. F., U. S. Patent 2,438,855 (1948).
50. van der Meer, W. A., Dutch Patent 60,862 (1948).
51. Sichel, Ferdinand, A. G., German Patent App. No. S-124,622 (1936).
52. Deutsch, P. A., *Food Inds.,* **19**, 1648 (1947).
53. Kerr, R. W., British Patent 533,023 (1938).
54. Glabe, E. F., *Trans., Am. Assoc. Cereal Chemists,* **2**, 20 (1943).

CHAPTER XXI

USE OF STARCH PRODUCTS IN THE TEXTILE INDUSTRY*

1. Introduction. The principal uses of starch and starch products in the textile industry are for sizing yarns (warp sizing) preparatory to the weaving operations, for sizing or "finishing" the cloth after the yarn is woven, and for printing certain types of fabrics with printing pastes which are frequently a mixture of dye and starch paste. The function of the starch product in warp sizing is primarily to protect the yarn so that it may be woven with less damage. After weaving, the size is generally removed. The woven goods are finished by a variety of processes either to make their appearances attractive or to prepare them for a particular commercial use. The function of starch products in finishing will vary with the purpose of the finishing operation. Color patterns are reproduced on some types of fabrics, as in calico, by a printing process. The function of a starch product in this operation is to thicken the solution of the dye and to act as a carrier for the color. Although each of these major applications of starch to textile manufacture is outlined, the following discussion is concerned primarily with warp sizing. This emphasis is intended to reflect the proportionate advancement which has been made in a study of the subject of warp sizing in recent years by the combined efforts of the carbohydrate chemists and textile technologists.

Warp sizing may be defined as the application of a protective coating or dressing to warp yarns prior to weaving. The purpose of the process is twofold: (*a*) to increase the weaving efficiency of the warp by reducing the number of warp breaks and the amount of shedding in the loom, especially with cotton and other spun yarns; (*b*) to improve the quality of the fabric produced by reducing the number of defects, such as knots arising from warp breaks, and, in smooth fabrics, produced from continuous filament bright rayon yarn, by preventing delustering of the yarn by abrasion on the loom parts. The process of warp sizing is also known as "warp slashing" or simply as "slashing," terms which are not descriptive and whose derivation is not clear. The general process is the same although the actual details of the procedure may vary widely according to the system used. For ease in description, the silk system, widely used on continuous filament rayon, is given below in some detail and the variations of the other systems noted briefly.

Warp sizing of continuous filament rayon is carried out on a rayon slasher, a machine which consists essentially of (*a*) a steam-jacketed size box or trough containing an immersion roll and a set of quetsch rolls. The latter, usually three in number, are power-driven and are arranged vertically one above the other. The lowest roll is partially immersed in the size liquor. The function of these rolls is to pull the warp from the warp beam in back of the slasher into the size box, and to squeeze the excess size liquor from the warp before the latter goes to the drying cylinders; (*b*) a set of steam-heated, power-driven drying cylinders, known as "dry cans," usually three, five, or seven in number; (*c*) a wrap-up mechanism consisting of a beam driven at the appropriate speed so the dried warp is wound up under the proper degree of tension. A press roll riding on the warp as it is wound on the beam helps to insure uniformity during this operation.

---

* The section on Warp Sizing was contributed by Dr. W. E. C. Yelland.

A warp consists of a definite number of warp yarns or "ends," usually about 2000 to 8000, depending on the construction of the ultimate fabric. These are arranged in parallel and are wound on a warp beam. In the slashing operation this warp is drawn from a single beam in back of the slasher over a drag-roll into the size box by the nip of the quetsch rolls. In the size box, the yarn picks up the size liquor either by passing through it under an immersion roll or merely by passing through the nip of the two lower quetsch rolls and being wet by the size carried up to the nip by the lowest roll as it revolves partially immersed in the size. There are several ways in which a warp may be threaded through the quetsch rolls, depending on such factors as the type of yarn, the number of ends in the warp, the degree to which the yarn is to be stretched during slashing, etc.

A detailed description of the operation of the quetsch (and of rayon warp slashing in general) is given by Mauersberger and Schwarz (1).

After leaving the size box and quetsch rolls, the wet warp is dried first on one side then on the other by passing over and under the alternate drying cylinders. Steam pressure is maintained in these cylinders such that their surface temperatures are in the range of 100-212°F. The actual temperature is determined by the drying requirements of the warp; *i.e.*, the number of ends, and the degree to which the excess size liquor is squeezed out by the quetsch rolls, the slashing speed, and the preference of the operator. On a multiple can slasher it is customary to operate the machine so that the maximum temperature is attained on the middle cans, while the first and last cans are cooler. This prevents an explosively rapid drying of the yarn on the can nearest the size box and allows the warp to leave the last can at a lower temperature.

Control of the actual "regain" (*i.e.*, of moisture content to which the yarn is dried) is ordinarily by manual control of the speed at which the warp is sized and passes over the drying cans. This depends on the experience and skill of the operator. The latter, in order to avoid underdrying of the yarn and consequent danger of mildew development, usually dries the warp to a moisture content lower than normal for the yarn. The size must therefore be of such nature that it is not decomposed or rendered insoluble by such overdrying. Recently, mechanical control of moisture has been introduced in some mills.

The warp, after leaving the drying cylinders, is wound up directly on a loom beam at the front end of the slasher. This beam is driven at such a speed that the warp is wound up under sufficient tension to produce a firm uniform beam with no loose or slack ends. Too much tension at this point is undesirable, as it is one cause of excessive elongation or stretch of the yarn during drying. During the slashing operation, it is usually considered desirable to keep the total elongation of the yarn at a minimum, certainly not over 5%.

After the warp leaves the final drying can and before it is wound on the loom beam, the individual warp ends, which have a tendency to be glued together by the size, are separated from one another for an instant by a series of iron bars known as split-rods or lease-rods. After this operation the sized and dry warp yarns should have no tendency to stick together again, no matter how tightly they are packed on the loom beam. A size which has little adhesion to the yarn will flake off, or shed, at the lease-rods. On the other hand, a size which binds the yarns too firmly together will cause trouble here in the form of warp end breakage. The yarns should separate readily and smoothly with a minimum amount of shedding at the lease-rods.

The lease-rods also aid in the insertion of lease strings in warps sized on the silk system. These strings keep the yarns in the proper relative order so they do not become crossed or snarled.

The beam of sized yarn is removed from the front of the slasher and placed in the loom. The ends are "drawn in" the loom, or else "tied in" at the end of a warp of the same number of ends which is just running out. The warp is then ready for weaving.

Slashers are operated at speeds varying from 20 to 60 yards per minute. The trend in modern slasher design has been consistently toward higher speeds.

In the silk system of slashing the size liquor is usually prepared in copper size kettles in batches of 40 to 100 gals. or larger, depending on the equipment available. For heating purposes the kettles may be steam-jacketed or may contain open or closed steam coils. The method of stirring may be manual or by motor-driven paddles. The following procedure is common. Water at ordinary temperatures is admitted to the size kettle up to about one-half or two-thirds of the final volume of the batch. The weighed amount of dry starch is added slowly with stirring until a uniform suspension or milk is formed. The temperature is raised by steam to approximately 212°F. and held there until the starch is gelatinized. Untreated or thick boiling starch used in cotton sizing may require heating for 15 to 30 minutes or longer at the boiling temperature. Dextrins and thin boiling starch require much less heating time. When gelatinization is complete, the paste is allowed to cool to 160-180°F. and the other ingredients, such as softeners, plasticizers, penetrants, etc., are added. The volume is brought up to the value desired by the addition of cold water. The size is then ready for use.

The above procedure assures that each size mix will have approximately the same concentration of dry substance and prevents an overdilution by condensation from the steam line, if open coils are used, or loss of water by evaporation when closed steam coils are used for heating.

Heating by injection of steam from open coils also has the effect of very vigorous mechanical stirring and may decrease the viscosity.

The prepared size may be stored in the size kettle, in storage tanks, or it may be fed directly to the slasher.

The kettles are frequently located on a raised platform so the size flows by gravity from the kettle to the size box. As the size is used up by the warp, more is admitted through a valve which is either controlled manually by the operator or, in the newest slashers, by an automatic size level control.

Where several slashers are using the same size liquor, it may be kept circulating through a main line from which the slashers draw it according to their requirements. The size storage tanks and pipe lines are usually not heated or insulated, so the size may cool almost to room temperature before it reaches the size box.

The temperature at which the size liquor is maintained in the size box varies with the type of yarn sized. For rayon it is usually 140-155°F. Acetates are run at lower temperatures. For cotton yarns, the temperatures are considerably higher, 190-210°F.

When spun rayon or cotton warps are sized on the cotton system there are certain differences in the procedure, although the principle is the same. The warp is drawn, not from a single beam, but from a number of beams placed in a creel in back of the slasher. The ends from the individual beams are combined into a single sheet of parallel yarns before passing into the size box. The size box of the cotton slashed is usually larger (40 gals. as compared to 10 gals. capacity) than that of the rayon slasher. It may contain two sets of squeeze rolls rather than a single set of quetsch rolls. The drying cans on a cotton slasher are usually fewer in number but considerably larger than those on a rayon slasher. There are also certain differences in which the leases are inserted at the beginning and end of the warps.

The equipment and procedures used in cotton warp slashing are described in considerable detail by Merrill *et al.* (*2*).

The principle of sizing woolen and worsted yarns is the same as that in spun rayon and cotton sizing, the chief purpose of sizing being to lay the surface fiber and thus to convert a fuzzy, hairy yarn into a smooth one which will not chafe or shed fiber in the loom. The equipment is analogous to that used in the cotton system, although a drying chamber usually performs the function of the drying cylinders in cotton and rayon sizing. Only a very limited number of rayon slashers use air drying in place of cylinders. Worsted and woolen warp slashing is discussed in detail by von Bergen and Mauersberger (3).

2. **Requirements of a Warp Size.** The properties which a satisfactory warp size must have may be classified under two general headings, (*a*) paste characteristics and (*b*) film characteristics. The former are those properties which govern the behavior of the size during preparation and application; the latter affect its behavior during weaving and subsequently in desizing.

a. Paste Characteristics. Among paste characteristics may be listed the following requisites: (*1*) ease of preparation, (*2*) uniform viscosity, (*3*) absence of pronounced congealing and skinning properties, (*4*) pH near neutral, (*5*) absence of foaming properties, (*6*) absence of prolonged tackiness during drying, (*7*) compatibility with other components of the size mixture, and (*8*) stability towards decomposition.

(*1*) Size mixes are readily prepared from starches, as the latter require no preliminary soaking or swelling treatment such as may be needed with gelatin or glue sizes. If the starch is added at a reasonable rate with moderate stirring to water at room temperature, a uniform dispersion is formed. Since most starches wet out readily, there is little difficulty due to the formation of aggregates or clumps. Dextrins and very thin boiling starches form pastes very easily if the supension is heated to 190-212°F. for 5 to 10 minutes. Thick boiling starches and gums require longer periods of heating at a temperature as close to the boiling point as possible. Although the viscosity of starch pastes may be reduced by prolonged heating, there appear to be no other effects which are undesirable.

(*2*) After the size paste has been prepared, the viscosity of the mix should not be readily diminished by further heating or by mechanical agitation, such as may be encountered in size circulation systems and even in the size box itself. This is important, as it has been shown that changes in viscosity affect the amount of size picked up by the yarn (4). In starches of high fluidity and dextrins most of the vesicle structure of the starch grain is destroyed during the pasting operation. Consequently the viscosity of size mixes prepared from these materials is usually low, and further changes do not take place even with severe mechanical agitation. A comparison by Katz of the relative viscosities of pastes of various commercial starches has demonstrated that corn, wheat, and sago starches produce pastes which are much less affected by heating and stirring than those made from potato and tapioca (5). The effect of these factors on the viscosities of thin boiling corn and sago starch pastes is also discussed by the same author (6).

(*3*) The size may cool considerably in the storage tank or in the pipe lines to the size box. This should not cause the size to form a permanent gel which cannot be redispersed readily on warming, otherwise lumps of gel may be picked up by the warp in the size box. These gel particles dry to hard spots which may cause a number of yarns to break out at once at the lease-rods, or may result in irregularities in the woven fabric. Thin boiling starches have less tendency to gel than native starches, and the tendency decreases with the extent to which the starch has been modified. Dextrins and very thin boiling starches form pastes of low viscosity which have practically no gelation tendencies at the concentrations at which they are used.

A phenomenon which may be related to gel formation is the formation of a skin or film of congealed paste on the surface of the size mix. It occurs when considerable evaporation may take place from the exposed surface of the size paste, for example in the corners of the size box, or in uncovered storage tanks where there is no stirring. These pieces of congealed size cause "hard size" spots on the warp and lead to the difficulties noted above. Dextrins do not exhibit this undesirable property to any appreciable extent. However, pastes of native starches, thin boiling starches, and gums are apt to form skins to a greater or less extent.

(*4*) The pH of the size mix should be preferably in the range near 6 to 7. Sizes which are alkaline are apt to promote foam formation, as the flannel blankets on the quetsch rolls produce small bubbles which quickly build up to large amounts of foam especially at high slashing speeds. This foam is troublesome as it may fill the size box and conceal the true liquid level. The latter, whether controlled either manually or automatically, may drop so far that there is not enough size liquor provided in the box. Then the yarn does not pick up sufficient size and the result is a "soft warp" with poor weaving qualities.

Excessively high acidities are harmful to the yarn, as they are apt to lower the tensile strength, especially of viscose and acetate rayons. Pastes made from starches and dextrins frequently are as high in acidity as pH 3 to 4. Materials intended for warp sizing are therefore often buffered to adjust the paste to about pH 6, by the addition of very small amounts of borax or sodium carbonate to the dry starch before it is sold. These added materials usually also promote the film-forming properties of the size.

(*5*) The size should not contain any material which would promote foam formation. Starches and starch products, unless buffered to above pH 7 show little tendency to foam. The tendency is further inhibited by the presence in the size mix of the oils and fats (usually sulfonated) which are added as softeners and plasticizers for the size film.

(*6*) As the size film dries on the yarn it should pass very quickly through the tacky stage, otherwise it is apt to stick the yarn to the drying cylinders causing the "ends" to break and wrap around the cylinder. Dextrins that contain a high percentage of cold water-soluble material may exhibit this phenomenon more than other starch products. However, they are not used to a large extent in warp sizing as their film characteristics are not usually satisfactory.

(*7*) The water with which the size paste is prepared may be of varying degrees of hardness, depending on the district in which the mill is located. Usually no effort is made to soften this water prior to slashing. The size should not therefore contain any materials which will react with the salts in the water to form insoluble materials which may be precipitated on the yarn. These would not be readily removed in desizing processes and would result in uneven dyeing. Starches give little trouble in this respect.

The size should also be compatible with materials, such as oils, fats, and their sulfonated derivatives, waxes, glycerols, and other humectants, pine oils, and similar penetrants which may be added to the size mix for specific purposes. Some of these may cause an increase in viscosity of pastes made from alkali gums, but otherwise little difficulty is encountered.

(*8*) Since the size liquor is prepared in batches, it is often used up within a few hours but it is not usual for the size to be stored for long periods of time. Therefore, a size mix should show as little tendency as possible to undergo decomposition under slasher room storage conditions. Sizes containing glue and gelatin materials may be less stable toward decomposition than starch sizes, and consequently preservatives such as cresylic acid or its derivatives are often incorporated in their mixes.

b. Film Characteristics. The size must be essentially a film-forming material and the film should possess to some extent the following properties: (*1*) tensile strength, (*2*) adhesion to the yarn, (*3*) flexibility and folding endurance, (*4*) hardness, especially resistance to abrasion, (*5*) insensitivity to overdrying, (*6*) moderate insensitivity to changes in relative humidity, (*7*) little tendency to develop static electrical charges on abrasion, and (*8*) ready resolubility regardless of the age of the size film, for ease in desizing.

(*1*) Although the tensile strength and the elongation of films of sizing materials have been studied in some detail by Neal (7), Farrow (8), Furry (9), and others, no well defined correlation has been found between these properties and the weaving properties of a sized warp. It is highly probable that no simple relationship exists between any single film property and the behavior of the film on the yarn in the loom. When the results of work now in progress (10) are available for publication, it is indeed likely that the relationship between film properties and weaving efficiency will be considerably more clearly defined. In general, materials which form films with sufficient tensile strength to be self-sustaining may be considered as possible warp sizes if their other film and paste properties are satisfactory. One exception to this conclusion is a reported use of a linseed oil derivative which has been been employed in Europe especially for sizing continuous filament rayon. However, it has not been used to any appreciable extent in the United States.

(*2*) Whether the size is applied as a rather heavy coating on the outside of the yarn, as for example on cotton, spun rayon, and other hairy yarns, or whether the size is present either in the form of a thin film lying mainly between the filaments or in the form of a matrix in which the filaments are embedded, as in continuous filament rayon sizing, the size film must adhere tenaciously to the filament and fiber surfaces. If the size film lacks this property, it will flake off or "shed" from the yarn at the split-rods at the front of the slasher, and in the loom at the dropwires, heddles, and reed, where the yarn is abraded and flexed by a contact with the loom parts. This loss of size exposes the yarn to the very abrasion from which the size is intended to protect it and a low weaving efficiency results. Starch films adhere well to the fiber surfaces of wool, cotton, and viscose rayon, both the bright and delustered varieties. However, as yet no starch product has been developed which by itself has the requisite adhesion to the filament surfaces of acetate rayon, nylon, and vinyon yarns to provide satisfactory sizing. The development of such a starch product or other materials which will function to anchor the starch film to the surface of the filament is as yet an open field for study.

(*3*) During the weaving operation the yarn is flexed rapidly and repeatedly at sharp angles. Consequently, the size film must be flexible enough and have sufficient folding endurance to enable it to bend with the yarn. A study by Furry (9) of films of materials which are used in sizing and finishing has shown that a series of seven starches can be listed in the order of a decreasing endurance to folding as follows: canna, sweet potato, potato, corn, rice, wheat, and dasheen. Film flexibility is usually increased by the addition of so called plasticizers and softeners. These are materials such as oils, fats, waxes, and, when greater ease of removal from the yarn is essential, the sulfonated derivatives of these materials. The amount of softener used depends entirely on local conditions of sizing and weaving which vary from mill to mill. These conditions involve such diverse factors as the type of yarn sized, the construction of the fabric to be woven (*i.e.*, the number of warp and filling yarns per inch), and the type and age of the looms in the weave room. In general, for starch sizes it is desirable to maintain the content of softener at an amount not exceeding 5% of the starch content of the size, otherwise film properties, such as resistance to abrasion, will be impaired.

(*4*) Little information has been available on the relation of film hardness and resistance to abrasion to the weaving efficiency of the sized yarn. It has been generally known that an excess of softener in the size will produce a size film which is soft and mushy in character and which will not protect the yarn against abrasion in the loom. The operator is always confronted with the dilemma of employing enough softener to produce a flexible sized yarn, without, however, adding so much softener that the yarn becomes soft and fuzzes badly in the loom. In the past, the solution of this problem has been by trial and error. The research now in progress under the auspices of the Textile Research Institute (10) is expected to develop valuable information on the relation of film hardness of sizing materials to the behavior of the sized warps during weaving.

(*5*) It has been pointed out above in the discussion of slashing that, in general, warps tend to be overdried as they leave the slasher. It is important, therefore, that the size be of such a nature that it is not readily affected by such abuse. Starch materials may tend to undergo a slight degree of conversion to gums and dextrins when they are overdried. This effect is not serious, as the materials produced in this manner are usually even more readily soluble than the original starch products. Sizes containing protein may, however, be rendered less soluble by such treatment.

(*6*) The air in the weave rooms is humidified in order to maintain desirable yarn properties, particularly those which promote good weaving. In weave rooms where viscose rayon is woven the relative humidity is generally maintained in the lower portion of the range 55 to 65%; for acetate rayon and cotton the relative humidity is considerably higher. However, a close control of the relative humidity is usually not possible over the extended period of time necessary to weave a single warp and the humidity may vary considerably, especially when the weave room is shut down, as it usually is under normal conditions over week-ends. The size film should be of such a material that its properties are not too grossly affected by a wide variation in the relative humidity; that is, it should not become tacky at a high humidity, nor brittle at a low humidity. Starches are analogous to other carbohydrate materials, such as cellulose, in their behavior under various conditions of humidity. They are softer and more flexible at high than at low relative humidity. Consequently the behavior of the yarn, in so far as atmospheric moisture is concerned, is not changed greatly by sizing with starch products. Only with a few types of yarns is it considered necessary to increase the humectant properties of starch sizes by the addition of hygroscopic materials such as glycerol, sorbitol, etc. Sizes for cotton are most apt to contain such materials.

(*7*) Since the process of weaving involves considerable friction, yarn against yarn and yarn against metal, there is an opportunity for the development of static electricity. This effect is not generally a problem, except with acetate rayon. In weaving acetate yarn, the filaments may become so highly charged as to repel each other. This may cause defects in the woven fabrics when a highly regular, smooth surface is desired. The size material should be of such a nature that it inhibits rather than contributes to the development of a static charge during weaving. Starch sizes appear to be satisfactory in that they do not cause static during the weaving of viscose rayon and cotton. As they do not have a high degree of adhesion to acetate yarn they are not usually employed for sizing acetate rayon. In cases in which they have been tried as sizes for acetate, the starches did not appear to affect the development of the static charge in any way. Gelatin sizes also do not appear to inhibit the formation of static electricity during the weaving of acetate yarn. The addition of humectants to the size and the use of high humidity during weaving tend to reduce the tendency of acetate warps to develop static charges during weaving.

(*8*) Very few fabrics are sold in the state in which they come from the loom. They are usually desized, dyed, and finished in subsequent operations. A few exceptions are denims, plaid, and figured materials, which are woven from yarns previously dyed. The warp size must be removed prior to dyeing, or else uneven dyeing and dye-resistant marks usually occur. The ease with which the size film is resolubilized is therefore an important size property, especially in respect to the processing of rayon fabrics. Cotton goods can be given a vigorous desizing treatment, but rayons, because of their low tensile strength when wet must be handled more carefully and cannot be subjected to the action of strong alkalies or acids. It would be desirable if the size were so readily solubilized that it could be removed from the fabric merely by rinsing the goods in warm water containing a small amount of soap to remove traces of oil and grease which the fabric may have picked up accidentally. Although some dextrins are desized by very mild soap and hot water (a "boil-off"), many finishers prefer to use an enzyme treatment to insure a complete removal of the starch in a shorter time and with less possibility of damage to the fabric. The use of enzymes for solubilizing starches in textile operations is too extensive in subject matter to be considered in detail here.* The warp is usually immersed in a solution of the enzyme (about 5% concentration) and allowed to remain in contact with this solution at the optimum temperature for the activity of the enzyme until tests on small swatches of the cloth no longer show the typical blue coloration given by starch and iodine. The enzyme and the solubilized size materials are rinsed from the fabric which is then submitted to the dyeing and finishing operations. The temperature at which each enzyme functions best is a characteristic of the enzyme and specific directions for its use are best obtained from the manufacturer. Many are active in the temperature range of 50-60°C. Too high a temperature inactivates the enzyme; too low a temperature greatly extends the time required for desizing. The time required for the complete removal of the starch product varies with the amount and type of starch on the yarn and on the particular enzyme used. A period of 30 minutes to 1 hour is usually sufficient.

The enzymes used in desizing may be simply diastatic in action, such as Diastafor, or they may also exert a proteolytic action, as for example, Degomma. Their sources are diversified, including various animal glands, malt, bacteria, and molds. Among the commercial enzymes commonly used are the following: Degomma, Diastofor, Exsize, Polidase, Polyzime, Rapidase, Serizyme, and Wiazyme. Also, Arcy is extensively used in the textile industry but more frequently it is used to convert native starch in preparation of the size.

Other materials which are not enzymes but which react with starch to solubilize size films are often used. An example of such a reagent is Aktivin, the sodium salt of toluene chlorosulfonamide. This compound oxidizes the starch to soluble and readily removable substances. It also exerts a bleaching effect on the yarn.

3. **Sizing of Continuous Filament Yarn.** The problems arising in the sizing and weaving of continuous filament yarns, such as viscose and acetate rayon, vinyon, etc., differ markedly from the problems presented in sizing spun yarns such as wool, cotton, and spun rayon and acetate. In the first group the yarns are smooth, the filaments continuous, and the yarns have just enough twist (usually 3 to 5 turns per inch) to hold the filaments together. In sizing these yarns it is not necessary to make the yarns smoother,

---

*See Chapters XV and XVI.

but only to bind the filaments lightly together in order to prevent a broken filament from being pushed back along the yarn to form a "fuzz-ball" or "bird's nest" as it is frequently called. Also, another purpose of sizing is to protect the outside filaments from delustering by the abrasion on the loom parts.

Gelatin and gelatin glues were formerly used almost entirely on continuous filament rayon. More recently, however, it has been found that white dextrins, both corn and tapioca, can be used satisfactorily as sizes for viscose rayon yarn. Dextrins from potato and sweet potato have been experimented with sporadically but they are not extensively used at present.

Although films of dextrinized starches are not as strong and flexible as films of the original starches from which they are derived, the dextrins, unless they are too highly converted, have sufficient film strength to bind the filaments together satisfactorily for good weaving. The dextrins have the advantage in that they are much more readily desized than the native starches. It is not advisable to try to give specific sizing formulations, as the conditions for sizing and weaving vary so much from mill to mill that a formula found to work successfully in one place often cannot be used in another without extensive modification. The size is usually applied from a mixture which possesses a low viscosity and contains from 35 to 50 lbs. of dextrin per 100 gals. of finished size. The amount of size which is picked up by the yarn may vary considerably, but the usual range is about 4 to 7% of dry size on the yarn and 5% is a common value. Generally a softening agent is employed with the dextrin, such as a readily dispersible sulfonated oil or tallow. The softener is used in amounts less than 5% of the weight of the dextrin. The size box is maintained at a temperature in the range of 140-165°F. The preparation of the size follows the general procedure given previously. A heating period at 190°F. for 15 minutes is sufficient to disperse the dextrin completely. Mixtures of dextrins and gelatin, or gelatin glue products have been used satisfactorily in the sizing of viscose rayon. In general, highly converted dextrins, such as the canary yellow type, have not proved successful for viscose rayon. These materials appear to pass through a prolonged tacky stage when they are cooked and dried and consequently may give trouble by causing the yarn to stick to the drying cylinders on the slasher. These highly colored dextrins may also impart undesirable tints to the yarn which are not removed in the desizing process. By themselves, no starch products, including dextrins, have shown sufficient adhesion to the yarn surfaces to provide for a satisfactory weaving of acetate rayon, nylon, and vinyon. However, dextrins are frequently incorporated in gelatin and gelatin glue sizes for sizing acetate yarns.

4. **Sizing of Spun Yarn.** The successful application of a size to spun yarn requires that a hairy yarn be converted into a smooth one, with as few projecting filaments and as little loss in flexibility as possible. This requires size mixtures of comparatively high viscosity. Size mixtures, such as those which work satisfactorily with continuous filament yarns merely saturate the porous spun yarns and on drying leave them stiffer than before sizing but still as rough and fuzzy. Many reports have been written on the problem to determine whether the size should penetrate into the interstices between the filaments or simply coat the surface of the entire yarn. However, satisfactory weaving has been achieved with warps on which the size is largely on the yarn surface and has only penetrated far enough to anchor this surface film securely to the yarn. Too much penetration of the size into the interior of the yarn appears to stiffen the yarn unduly.

Native, or unmodified corn starch was formerly used to a large extent to size cotton yarn. Thin boiling or modified starches were developed more recently in order to provide products which have a lower viscosity for the same amount of dry substance in the size and which have less tendency to form gels in the size box and pipe lines supplying the size when the temperature of the size is lowered. In conjunction with a small amount of softener, thin boiling corn starches are perhaps the most widely used sizing material for cotton warps. The concentration of size varies considerably, depending on local conditions. About 0.5 to 1.0 lb. of dry size per finished gallon is a range of concentration frequently encountered in practice. The amount of dry size added to the yarn may be in the neighborhood of 8 to 10%. The softener is employed in varying amounts up to 5% of the weight of the starch. The former is usually a tallow or wax or occasionally a sulfonated derivative of the fat. Thin boiling starches which have been manufactured by an acid modification and those made by an enzymic conversion are the types most commonly used. Oxidized starches have not been extensively used for the sizing of cotton warps.

In so far as sizing and weaving alone are concerned, acid-modified, thin boiling starches would, no doubt, be satisfactory for sizing viscose rayon spun yarns. However, the ease of removal of sizes made from these thin boiling starches is not sufficiently great to permit their general use. They require a desizing treatment which might be injurious to rayon. Instead, starch gums, the so called British gums, are used for this purpose to a large extent. These gums form pastes which exhibit high viscosities, comparable in this respect to the native starches or to thin boiling starches of low fluidity. However, the films formed from British gums are considerably more readily dispersed, and, consequently, warps which are sized with them can be readily desized by a mild enzyme treatment. A size concentration of 0.5 to 0.6 lb. per gallon is commonly used in practice. This concentration is normally sufficient under ordinary sizing conditions to allow 6 to 8% of size to be taken up by the yarn. Highly dispersible, sulfonated oils are used as softeners. Frequently additional materials, such as pine oil, are added in small amounts as penetrating agents, especially when the spun yarn is a blend of viscose and acetate fibers. For these fiber blends many size mixtures have incorporated in them considerable quantities of gelatin or gelatin glue; the more the acetate in the blend, the higher is the percentage of gelatin used in the size mixture. For sizing spun yarns, especially cotton as noted above, some mills prefer to make their own thin boiling starches from native starch by treating the starch paste with an enzyme prior to sizing. Other starch-modifying agents, such as Aktivin, are preferred by some mills. Conversion of starch at the textile mill is a procedure which requires considerable skill and careful control in order to achieve uniform results. Properly executed, however, this procedure appears to produce satisfactory sizes.

Thin boiling corn starches of low fluidity find considerable use in the sizing of warps of single ply worsted or woolen yarn. Plied, or highly twisted woolen yarn, is frequently woven without size.

The rapid development of the synthetic fiber industry in the past few years has stimulated considerable research and investigation of the methods and materials used in sizing, especially rayon sizing. Many mechanical improvements have been developed which have increased the sizing speeds and which have led to a closer control of the process mechanically. Materials for sizing have been improved so as to provide sizes which are applicable not only to single types of fibers, but to a variety of blends of natural and synthetic fibers.

5. Finishing Textiles with Starch Size. The principal function of starch in finishing textile fabrics is to impart or accentuate the desired physical characteristics in the cloth. When the large variety of fabrics which are manufactured is considered, it may be appreciated that many modifications of starch are required to obtain the various results desired.

Starch was originally used primarily as a stiffening agent for fabrics; that is, its function in this case is to increase the structural strength of the yarn and to lock the yarns in a fixed position with relation to each other in the fabric. When no other characteristic is desired except possibly to add weight to the fabric, an unmodified starch, such as corn starch, is suitable for the purpose. Thus, unmodified starch is suitable for sizing certain types of gauze, netting, cloth used to cover hat frames, and some of the other loosely woven fabrics.

If in addition to "yarn fixation" the added characteristic of flexibility is required, the starch is modified by procedures which will give the combined results desired. For example, a flexible finish is required for stiffening dress goods such as organdies so that apparel made from the fabric will not readily crease when it is worn. The added property of flexibility is usually obtained by procedures which reduce the gel-forming properties and, in general, the viscosity or body of the starch paste. As a result of this modification, however, more starch (weight of starch to fabric) is required to produce an equivalent amount of stiffening action. Frequently added weight is an advantage, since fabrics may be sold on the basis of weight rather than yardage.

One of the more recent developments in the art of finishing textile fabrics, known as sanforizing, involves an unusual combination of the effects described. In this operation the goods are shrunk by the application of moisture and hot irons and the yarns are "fixed" by the application of a starch size. However, a flexible rather than a "board-like" finish is usually required for most of the fabrics which are sanforized. Highly converted corn starches (acid-modified to a fluidity of 90), chlorinated corn starch, and dextrins are commonly used to perform the two principal functions of the size in this finishing operation. In addition to the other properties of starch which are required (depending on the type of fabric sanforized) the starch product should not produce a size which, after application, will stick to and tend to caramelize on the hot sanforizing irons.

In general, a flexible finish is more often desired for fabrics than a harsh, "board-like" finish. Even in instances in which a high degree of stiffening is required, flexibility may be essential so that when the fabric is folded, as in the manufacture of shirts and collars, the yarns will not be broken or injured. Therefore, starch products which set to soft gels when gelatinized are normally preferred. It is a natural consequence that the non-cereal starches, particularly potato, should have been used to a large extent in finishing. One of the principal disadvantages of the use of potato starch is that its paste viscosity is not stable; that is, the viscosity tends to decrease when the paste is boiled or held at elevated temperatures or stirred vigorously. This instability is magnified in alkaline media and it is a common practice to add borax to finishing sizes in order to increase the brilliancy of the finish. Therefore, since the results obtained in finishing are to a large extent related to the viscosity of the size, the use of potato starch leads to variable effects: the first part of a batch of the size gives results which are different from those obtained by the use of the later part of the batch. In order to maintain a greater degree of stability and also to produce a firmness of finish which cannot be obtained with unmodified potato starch alone, more recent practice involves the use of a potato starch which is modified (*e.g.*, with acid to a fluidity of 20) purposely to reduce its paste viscosity. Less apparent instability of paste viscosity results and the latter is accordingly, but erroneously, said to be "stabilized." In addition, owing principally to the greater weight of size which may now be added, some improvement in the firmness of the finish is obtained. Another preferred procedure is to add corn starch or sago flour to the potato starch in order to increase the paste stability and firmness imparted by these starches.

Wheat starch has been used extensively in finishing. Although sizes made from wheat starch do not possess the degree of instability in paste viscosity that is shown by sizes made from potato starch, they also do not impart the brilliancy and flexibility of finish which is obtained by the use of potato starch. Rather satisfactory finishes may be made by the use of corn starch gums and chlorinated corn starch products. For example, a size that is suitable for finishing fabrics such as twills and denim is made from corn starch by a procedure described by Kerr (11) in which the starch is allowed to be converted in the presence of chlorine gas until it has attained a paste viscosity corresponding to a fluidity of 10. Satisfactory products may also be made by the use of hypochlorite to modify the starch. Representatives, presumably, of this class of oxidized starches are sold under the trade names of Beatsol, Hercules gums, Clearsol, Clinco and Stayco products.

The general characteristics usually desired in finished fabrics are that they possess firmness and give the impression of solidity, which is usually described as "fullness." These characteristics are not to be confused with those of a weighted or filled cloth, the production of which is outlined in a following paragraph. The characteristics under discussion are difficult to define and are often referred to in the textile industry as the "feel" or "hand" of a fabric. Pierce (12) has discussed the "hand" of cloth as a measurable quantity and Dreby (13) gives physical methods for evaluating the "hand" of fabrics.

From these discussions it is apparent that all the results accomplished in the finishing operation cannot be obtained by the use of one starch product alone. Therefore, finishing sizes are usually made of a combination of starch products. To secure special sizing effects, the size formulation may contain adjuncts such as soaps, fats, sulfonated oils, and other materials which in many cases act as plasticizers for the applied size. The study of plasticizers for starch products assumes importance in this connection. In view of the development of many varieties of such agents in recent years, the immediate future should produce significant advances in the formulation of superior textile finishes from starch products.

Frequently no more is required of a finishing size than that it add to the weight of the fabric. Usually a highly converted starch product is used for this purpose, since to make the applied size less obvious, penetration of the size into the yarn is necessary. The penetration of a size is related to its viscosity. Therefore, a reduction in the viscosity of a starch product, as for example by acid hydrolysis, permits loading of the fabric with a high percentage weight of size and permits application so that the size is neither visible nor perceptible by the "feel" of the stiffening effect imparted by unmodified starch. In the extreme cases, such as the loading of goods for rough working clothes (over-alls), very highly converted acid-modified dextrins are used and as much as a 25% increase in the weight of the fabric may result. Sizes made from enzyme-converted starch are also used for the purpose of weighting fabrics.

In some cases the loading of goods is not primarily to increase the weight but to fill the interstices between the woven yarns. The back-filling of sheeting fabrics and of rug bases are two examples of this application of sizes. For this use an unmodified non-cereal starch such as potato or tapioca or a slightly modified starch can be used. For certain types of back-filling tapioca dextrins are used. For back-filling Wilton rug bases, for example, a tapioca dextrin made by enzymic conversion is considered to be a superior sizing agent. It is claimed that this product produces the flexibility and durability of surface which is required in a rug base.

In order to provide a complete filling of the fabric, to increase either its weight or its opacity, inorganic salts, clays, and other pigments may be incorporated in the starch size. As an example, window shade fabrics are filled with a size consisting primarily of clay, pigment, and either a corn starch dextrin, suitably plasticized, as the adhesive or a dextrin possessing a similar adhesive value and flexibility of a size film.

An additional characteristic required of a finishing size, particularly of those applied to dyed fabrics and to those made from synthetic polymers is that the size should be transparent after application and drying. Otherwise the color of the dyed fabric may be dulled or altered in shade or the luster of the goods might be impaired. Starches or starch modifications which show a pronounced tendency to retrograde* are, therefore, not as suitable in this respect as those starch products which do not. For this reason, oxidized corn starches and some types of gums and dextrins are preferred for finishing dyed goods or fabrics possessing a high sheen. No doubt in the future there will be an extension of enzyme technology to prepare suitable starch sizes for this and other special textile finishing operations.

6. **Printing Pastes.** Multicolored textile materials may be made by

---

*See Chapters VII and VIII.

printing designs on woven goods as well as by weaving fabrics with dyed yarn. The reproduction of colored patterns is usually accomplished by the use of engraved rollers and hence the art is termed printing, although some processes involve the use of stencils and air-pressure sprays. Printing pastes are made by mixing dyes and other necessary chemicals with a water solution or paste of starch, gum, or other suitable colloid. These are called thickeners or carriers. Starch products are widely used for this purpose.

The principal function of starch in printing pastes is to act as a carrier for the dye and the chemicals required to develop and fix the dye. Inasmuch as the colors are in most cases transferred from rollers, the paste of the starch product should possess the required body, plasticity, and other physical properties so that the printing paste will operate on the rolls. It should feed smoothly into the design on the rollers and remain in place until it is transferred to the cloth. Wheat starch and wheat starch products have been extensively used in the past, principally because of the excellent working qualities of their pastes. As the paste is transferred to the cloth, it should penetrate the yarns to the desired extent but it should not spread along the fibers to deform the pattern. Since the rollers apply the paste by a slight pressure, a limited amount of thixotropy is desirable, for as pressure is applied this effect favors the penetration of the color and as the applied pressure is removed, the color then tends to remain in position. Printing is applied to many fabrics which vary not only in their construction but also in the type of yarn from which they are woven; for example, fine silks, cotton, rayon, and acetate fabrics. Each of these variables affects the penetrability of the cloth, and an appropriate starch product is selected for printing, accordingly. The penetration of a paste depends largely on its viscosity; the lower its viscosity the better its penetrating power, but occasionally for some fabrics it becomes necessary to add a penetrant (*e.g.*, a glycol) even to a starch product of very low paste viscosity. The viscosity of the pastes of starch products is very materially altered by the pH of the medium and both acid and basic dyes are used in the printing of textiles. However, by far the greater portion of printing is done with vat dyes and an alkaline printing paste. Corn and some of the other cereal starch products are the most stable in an alkaline medium; the non-cereal starch products such as potato are the least stable. As a general rule, dextrins, and in particular the British gums, are more alkali-stable than their corresponding parent starch. In the printing of the better grade of fabrics, such as dress goods, it is desirable that the colored pattern completely penetrate the fabric. The color reproduction thereby simulates one woven into the fabric and in addition the colored design is less readily lost when the fabric is washed or otherwise cleaned. For less expensive fabrics, such as calico, and decorative fabrics, such as cretonne, a surface printing often suffices, indeed, is frequently preferred, since for the same amount of dye and printing paste used a better "color value" is obtained; the color is localized on the surface. In the former instance, highly modified starch products such as dextrins are used, whereas in the latter, unmodified starch or flour is satisfactory.

In many cases it is desirable to remove the starch or other colloid after the printing process. Dextrins and modified starches are naturally more readily removable by washing than unmodified starch. Dextrins are also preferable when the thickener is not removed in that their presence does not adversely affect the "feel" of the finished fabric to produce a hard, stiff finish. Starch is preferable when the full stiffening effect of the paste in the finished fabric is desirable. Also, when printing is done with pigments, the thickener is used to fix the color and in this case the ready removal of the starch product by washing is neither required nor desirable.

A disadvantage to the use of starch products as thickeners is that to obtain the proper paste consistency it is necessary to add as high as 15% of an unmodified starch or flour and a correspondingly larger amount of modified starch of lower paste viscosity to prepare the printing paste. A high content of solids in the printing paste leads to a poor "color value." Natural gums, such as tragacanth, form relatively thick pastes at a definitely lower concentration. However, their cost is many times that of starch. Accordingly, mixtures of a starch product and a natural gum have frequently been used in order to obtain a better "color value" than can be obtained with starch alone. The more recent practice has been the use of starches of superviscosity, prepared for this purpose; that is, the use of starch products of abnormally high paste viscosity so that materially less dry substance is necessary to produce the body which is required in a printing paste. In addition, these special starches for textile printing form pastes with excellent working characteristics and possess a paste viscosity which is relatively more stable under the adverse influences of acid, alkalinity, or other added chemical. The production of starches of high paste viscosity and a discussion of the lability of these starches in the presence of acid or alkali, as the case may be, are given in a previous chapter (Chapter VI). Recent developments in this connection may be noted in patents issued to Gill and McDonald (14), Griffen et. al. (15), Lienle and Adams (16) and Kerr (17).

BIBLIOGRAPHY

1. Mauersberger, H. R., and Schwarz, E. W. K., Rayon and Staple Fiber Handbook, New York, 232-245 (1939).
2. Merrill, G. R., Macormac, A. R., and Mauersberger, H. R., American Cotton Handbook, New York, 415-441 (1941).
3. von Bergen, W., and Mauersberger, H. R., American Wool Handbook, New York (1938).
4. Yelland, W. E. C., *Textile Research,* **10**, 343 (1940).
5. Katz, J. R., *Textile Research,* **9**, 114 (1939).
6. Katz, J. R., *Textile Research,* **9**, 146 (1939).
7. Neal, S. M., *J. Textile Inst.,* **15**, T443 (1924).
8. Farrow, F. D., in Walton, R. P., A Comprehensive Survey of Starch Chemistry, New York, 215 (1928).
9. Furry, M. S., *U.S. Dept. Agr. Tech. Bull., No.* **674** (1939).
10. Spun Rayon Warp Sizing Project Reports by the Textile Research Institute, Inc., at the National Bureau of Standards, Washington, D. C., and at the North Carolina State Textile School, Raleigh, North Carolina.
11. Kerr, R. W., U.S. Patent 2,108,862 (1938).
12. Pierce, F. T., *J. Textile Inst.,* **21**, T377 (1930).
13. Dreby, E. C., *Proc. Am. Assoc. Textile Chemists and Colorists,* 497 (Oct., 1942).
14. Gill, L. O., and McDonald, J. W., U.S. Patent 2,407,071 (1946).
15. Griffin, I. Truax, D. E., and Nuttall, N. H., U. S. Patent 2,413,320 (1946).
16. Lienle, R. H., and Adams, F. H., U.S. Patent 2,430,430 (1947).
17. Kerr, R. W., U.S. Patent 2,468,207 (1949).

CHAPTER XXII

STARCH ADHESIVES

ALEXANDER FRIEDEN

1. Introduction

a. Definitions. At the outset it should be realized that the preparation and use of adhesives have been inexact and very much a matter of rule of thumb. If the chemist working with adhesives is to master this art and science thoroughly, he must have a broad knowledge in many allied fields. He must know something of the chemistry of paper, wood, textiles, metals, and resins, since it is the surfaces of these materials which must be joined together. He should be familiar with the subject of molecular forces, of surface phenomena, and of interfaces. He must have a good understanding of colloidal behavior, plasticizers, wetting agents, and above all he needs to know the theoretical conceptions of adhesive substances and of adhesion.

The art of the preparation and use of adhesives is centuries old, although the science is very young, so young, in fact, that many of our present authorities on commercial adhesives fail to give recent theoretical studies sufficient consideration. Early editions of Thorpe's Dictionary of Applied Science define adhesives simply in the functional sense, as "substances or preparations of gummy or gelatinous character used for the purpose of joining together or effecting the mutual adhesion of surfaces of bodies." Brown and Truax (1) formulated a definition which takes into account theoretical considerations: "Any material which can be obtained as an emulsoid sol of suitable consistency which wets a particular surface and which subsequently forms a strong elastic jelly by cooling, heating, evaporation, or chemical reaction, must be regarded as a glue for that surface." This definition was formulated about 20 years ago. It is extremely significant that few theoretical studies on adhesives date back more than 25 years.

This chapter is limited to adhesives made from starch, a group of adhesives that has become extremely varied both in composition and in application. The importance of this group to the industry has grown steadily, so that now there are few manufacturing processes requiring adhesion in which starch adhesives are not used to a greater or lesser extent. The multiplicity of modifications and applications of starch adhesives will be shown as we progress with our discussion, though no special effort will be made in this chapter to give formulas. The purpose will be rather to show the rational bases for the different types of adhesives, and to point to the underlying principles, so that the student of adhesives may be able to analyze his needs in the light of fundamentals and deduce the direction in which lies the probable solution of his particular problem.

The scientist is often plagued by confusion in terminology. In the case of starch adhesives, there is considerable vagueness in connection with terms to be applied when starch or starch derivatives are treated with water, with or without heat. The resultant product is definitely not a solution, unless the starch decomposition has progressed so far that the end-product is a sugar, in which case it is no longer an adhesive in the accepted sense. As starch and starch products short of sugars are colloidal in character, colloidal nomenclatures should be used. Aqueous adhesives would therefore be hydrosols or hydrophilic dispersions (2). When the hydrosols become jelly-like, through evaporation, temperature changes, chemical reactions, etc., they are hydrogels. When completely dried out, they should, provided they still retain the property of redispersion in water, be known as xerogels, a term suggested by Freundlich (3). It is also desirable to utilize the term "peptization" for all cases in which a substance is dispersed in water to a hydrosol or hydrogel, rather than use the misleading term "solution."

b. Theoretical Consideration of Adhesives. Bancroft (4) considers that adhesion in general depends upon adsorption of the adhesive on the surfaces which are joined, and that the degree of adhesion can probably be measured by the amount of adsorption. Adsorption plays an important part in adhesion. It is, however, too inclusive, and to explain all adhesive phenomena by it is an oversimplification of the factors involved. It is now generally established that there are two types of adhesion; namely, specific and mechanical (5). Specific adhesion involves a force of attraction between the adhesive and the surface and is particularly noticeable for highly polished, smooth surfaces. Mechanical adhesion involves the imbedding or "keying" of the adhesive into the crevices of the porous material. Although mechanical adhesion is possible only with porous surfaces, specific adhesion is effective on surfaces that are non-porous as well as porous.

Bikerman (6) in discussing problems of adhesion illustrates graphically specific and mechanical adhesion, even though in an indirect way. If a crystal lattice is broken into two parts and the parts brought together in exactly the original position, the restored whole should show no break and be exactly like the original, as the known forces between the atoms and molecules are reversible. That this is not so is due to two factors: the fact that when the solid is fractured, the new surfaces, on exposure to the atmosphere, readily adsorb air molecules or react with oxygen or moisture of the air. Thus the fracture cannot be rejoined because one cushion of air is merely being pressed against the other. The adhesion of air is negligible, and the pieces fall apart. However, if this were the sole reason why a fracture could not be rejoined a vacuum could be used to give a bonding similar to the original, and such is not the case. There is, therefore, a second cause for this failure, which is that when a solid is broken the surfaces of separation are neither flat nor smooth, and when an attempt is made to put them together again it is impossible to place every protrusion on one surface exactly in the indentation of the surface from which it was broken. The surfaces touch each other only at a few points, and the total area of contact is a very small fraction of the area of contact before the fracture. Therefore, the force required to separate the rejoined fractured body is a very small fraction of that required to produce the fracture.

The adhesive material that will overcome the molecular film of contamination on a fractured surface does so through specific adhesion, and an adhesive which will fill out all the holes and crevices of the solid, thus establishing contact along the whole surface of the fracture, operates through mechanical adhesion.

Specific adhesion is produced by molecular forces. The results of a large number of quantitative tests on joints made with pure chemical substances as the adhesives (7) indicate that the fields of molecular attraction of the opposing surfaces are superimposed upon the ordinary cohesive forces between the molecules, provided the film is very thin, although the effective range of molecular attraction may be hundreds of molecular diameters. There also appears to be a close connection between adhesion and the structure of the molecule. Thus, in the study of pure chemical substances as adhesives between polished surfaces, the aromatic compounds have definitely more adhesion than those of an aliphatic nature (7). The molecular arrangement is also apparently a determining factor, and maximum strength may be expected with disorderly molecular arrangements (8).

Mechanical adhesion is produced by the "keying" of the adhesive in and around the fibers and the pores of the materials joined. There appears to be a considerable difference of opinion among investigators on the rôle of specific and mechanical adhesion in joints of porous surfaces. Clark (9) concludes from a study of the structure of glue films (gelatin) by x-rays that adhesion is a mechanical solidification of the gel around and upon minute fibers, because the adhering films of glue showed no property essentially different from the block of the material. On the other hand, de Bruyne (10) emphasizes the specific nature of glue joints. Brown and Truax (1) also feel that specific adhesion plays an important part in glue joints, such as those of wood. It is quite logical that in porous bodies mechanical predomirates over specific adhesion. We can, however, think of few cases of adhesion that could be considered mechanical only, since the nature of adhesive forces should be considered similar to chemical affinity. Without the chemical affinity between the adhesive and the surfaces, there would be no adhesion except that due to the physical "hooking" effect of the adhesive.

It was mentioned above that Bancroft considered adsorption to be the all important factor in adhesion. This was thought to be too inclusive. Von Buzāgh (11) has analyzed "adhesion through adsorption" in greater detail, at least as it functions in the case of adhesion of microscopic particles on a plate of the same substance. His conclusions are that adhesion is the result of the interaction of surface films and the adsorption layers. Any factor that will affect the properties of the surface films will affect the adhesion. Such factors are the chemical nature of the joined surfaces, molecular structures, size of units, etc. Of particular interest is his study of the effects on adhesion of added substances which tend to modify the structure and composition of the adsorption film, such as strong electrolytes, non-electrolytes, and colloids. The results show that the basic electrical forces operating in "adhesion through adsorption" are very much the same as those in specific adhesion.

The thickness of the adhesive film is extremely important in determining the strength of the bond. Schnurmann (12) has made an interesting theoretical study of the film factors involved in adhesion, and particularly in specific adhesion. Surfaces which are optically flat may neverthless have many irregularities. The optically flat surfaces employed by him had irregularities of the order of 10^{-5} cm. in height. Using metal and glass surfaces, he found that films of foreign matter as thin as 10^{-7} cm. in contact with the "high points" of the surfaces act as effective adhesives. The tenacity of a monomolecular layer of condensed water vapor is extremely high. To produce maximum adhesion, the liquid adhesive should be applied in sufficient quantity to fill out the irregularities and provide complete surface contact. Any increase in film thickness beyond that necessary for complete contact weakens the bond. The irregularities should be of a small order of magnitude, for it has been shown experimentally that the strength of the adhesive layer increases as it is reduced to a millionth of an inch or even less.

McBain and Lee (8), discussing the relationship of film thickness to strength, consider that the liquid adhesive is modified and immobilized by contact with the solid surfaces, so that either the range of the molecular attraction approaches the magnitude of the film thickness, or the molecules touching the solid surfaces become oriented, and through chain effect or through micellar linkages, extend this influence through the body of the liquid and subsequently into the solidified film.

Bikerman (13) in a study of paraffin wax joints between metals finds agreement with the general rule that thin joints are stronger than thick ones. He believes this effect to be due mainly to the probability of a weak spot in the thicker paraffin film and partly to the "graining" and possible crystal growth in the case of thicker paraffin films. Bikerman also brings up the point that the rate of evaporation will be affected by the thickness of the film and that this rate will in turn affect the structure and stress in the film. In a thin liquid film, the rate is more uniform, and less stress will be produced in the solidified film. In a thick film, a joint may fail within the adhesive film because the cohesive forces of the molecules of the film are not as strong as the forces causing strain.

McBain and Lee studied the strength of joints of varying film thickness formed by using metal surfaces and adhesive gums. The adhesive was allowed to melt completely to form a film, and the metal pieces were pressed together by hand. When aluminum plates and shellac were used, the values given in the accompanying tabulation were obtained.

Thickness of adhesive inches	Joint strength lbs. per sq. in.
Usual (pressed by hand)	2250
0.04	750
0.05	500
0.08	350
0.13	400
0.17	35

Another factor to be taken into consideration as affecting adhesion is the polarity or non-polarity of the adhesive and the surfaces to be adhered. If strips of polar and non-polar solids are immersed in polar and non-polar liquids, it will be observed that there is an attraction between the polar solid and the polar liquid, and between the non-polar solid and the non-polar liquid, but not between the polar substance and non-polar liquid, or the non-polar substance and the polar liquid. Thus if wood, which is normally polar, is immersed in water (polar) and benzol (non-polar), the interfaces will show an attraction between the water and the wood and none between the wood and the benzol. Similarly, if a plate of bakelite (non-polar) is immersed in the same solvents, the attraction will be between the bakelite and the benzol and not between the bakelite and the water.

De Bruyne (10) has stated as a basic rule of adhesion that with pure or simple substances strong joints can never be made between polar surfaces and non-polar adhesives, or between non-polar surfaces and polar adhesives.

Stamm and Seborg (14), using synthetic resins of decreasing polarity (that is, increasing state of polymerization) in measuring the shrinkage of glued wood joints, found that as the polarity of the resin decreased, the anti-shrinking efficiency of the adhesive also decreased. Since the shrinkage of wood is considered to be due mainly to free hydroxyl groups of the cellulose, which through hydration cause swelling and dimensional changes, it is thought that the more polar substances orient themselves in the structure so that the polar groups satisfy a greater proportion of the hydroxyl groups or points of sorption, and thus reduce the tendency to swell. There are, of course, other factors that come into play here, such as greater penetration of the smaller, less highly polymerized molecules into the wood, but it has been shown by these and other investigators that the chief factor is the polarity of the surfaces in contact. Of interest in this connection is the change or reduction in polarity that can be effected in cellulose (wood, paper, etc.) through heating. Apparently the neighboring polar hydroxyl groups form non-polar linkages at elevated temperatures. This is particularly well illustrated in the effect of moisture adsorption in paper (15). It is a fact that should be considered in the study of any problem on adhesives.

This brief theoretical discussion concerns principally molecular aggregates.

Starch adhesives are composed chiefly of colloidal units, *i.e.*, aggregates much larger than molecules. The active forces are, therefore, considerably more complicated, though basically similar. From a practical point of view, the following main factors should be considered in determining satisfactory starch adhesives.

1. *Colloidal Lability.* The property of an adhesive dispersion of starch to transform from a hydrosol to a hydrogel, and the great sensitivity of the reversible system, hydrosol $\rightleftharpoons$ hydrogel, to slight changes are fundamental to these adhesives. By a slight loss of water through absorption or evaporation, by the addition of certain chemicals, by a modification in the operating temperatures, etc., a hydrosol, which is fluid, can be changed into the jelly-like hydrogel. This change is accompanied by an increased rate of tack and better bond. In practice, the system must be adjusted to transform the hydrosol to the particular hydrogel within the tolerance of the operation and the requirements of a particular adhesive.

2. *Specific Bond.* Associated with colloidal lability is the requirement that the adhesive wet the surfaces to be adhered.

3. *Mechanical Bond.* This requires proper viscosity and flow, penetration, and cohesiveness of the adhesive.

4. *Type of Film.* The thickness and structure of the film are important factors.

5. *Deformability.* The adhesive must be able to retain its function in the face of unfavorable conditions. It must be capable of adaptation to film changes subsequent to setting, shrinking, shock, humidity changes, etc.

In connection with these requirements, it should be emphasized that the method of applying the adhesive is very often as important as the properties of the adhesive itself. The conditions for applying the adhesives, by rolls, wheels, sprays, or brush, etc., are quite different in each case, to say nothing of the different conditions with each machine and each plant (16). The experienced chemist, engineer, or operator may well be able to improve upon the operation of any type of adhesive through slight changes in the machine or the adhesive. In many cases, experience and ingenuity will coordinate machine and adhesive in such a way as to produce maximum efficiency at lowest cost.

No attempt will be made to give formulas for the different types of adhesives. Other chapters in this volume are concerned with the constitution of starch micelles and the behavior of these micelles when treated with water. For the purpose of the present discussion we shall simplify the micellar structure and consider it to be built up of two basic units; namely, glucose units combined in a molecular chain, and molecular chains united by primary and secondary valences to form the micelle.

Considering it thus, and keeping in mind, on the one hand, the numerous molecular chains that go into the building of a micelle and, on the other hand, the play of the primary and secondary valences in the spatial arrangement of these chains, we can see that in disrupting the micelles multitudinous fragments can be obtained, each differing in size and in the number of exposed active and potentially active groups. Between the extremes of the undisturbed micelle and the simple glucose units which serve as the basic building blocks any combination and fragmentation are possible. With the uninjured micelle as the starting material, any of the micellar fragments except the final sugar units, such as maltose or glucose, may serve as adhesives. In practice, the two extremes can be represented by starch itself at the upper extreme and by the thin dextrins, such as envelope dextrins, at the lower extreme. In between lies the entire range of other adhesives.

2. Types of Starch Adhesives.

a. Starch As an Adhesive. Gelatinization in Situ. The best illustration of the action of unmodified starch as an adhesive is its application in the form of a slurry with water to surfaces, heating to gelatinize the starch, and pressing together the starch-coated surfaces. The starch micelles combine with the water, swell, and burst to form a gelatinous mass producing an extremely strong bond. The process is generally known as "gelatinization *in situ*" and can be applied for bonding purposes to surfaces of paper, fabric, or to any surface that is wetted by an aqueous slurry, and to which heat can be applied. Modifying agents may be added to the slurry, which may serve both to improve the working characteristics of the adhesive and the resulting bond. Such agents may be defoamers, plasticizing agents, wetting agents, alkalies, etc.

Recently the method of gelatinization *in situ* has been perfected for use in the manufacture of corrugated and laminated board (17). The process is now widely used in this country and abroad and has replaced sodium silicate, which had been used heretofore in many plants. It is generally referred to as the "Stein Hall process." The essential details in connection with its use in the manufacture of corrugated board are described in the following communication from J. V. Bauer, the inventor of the process.

> Corrugated board is usually made by a continuous two step operation. It consists of corrugating a strip of paper by means of steam-heated fluted rolls, applying adhesive to the tips of the corrugations on one side, bringing a paper liner in contact with the adhesive-coated tips, and forming the bond with the assistance of heat and considerable pressure. This operation may be considered as the first step and forms what is known as single faced corrugated board, consisting of a corrugated strip of paper bonded to a smooth surfaced strip. This phase of the fabrication is performed on a machine known as a "Corrugator" or "Single Facer." The second step of the operation consists of applying an adhesive to the tips of the exposed corrugated surface, bringing a liner in contact with it, and forming the bond with the assistance of heat and just sufficient pressure to hold the paper in contact without deforming the corrugations. This second phase of the fabrication is performed on a machine known as the "Double Facer." The result of these two operations is a stiff paper board comprising two smooth outer paper surfaces bonded to an inner core of corrugated paper. The resultant corrugated board is cut into pieces of the desired length by means of a cut-off knife located at the discharge end of the "Double Facer."
>
> In the manufacture of corrugated board the adhesive problem is considerably more difficult than that of bonding two or more smooth surfaced strips of paper, as only a very moderate degree of pressure can be used to assist the formation of the bond between the single faced board and the second liner because of the danger of crushing the corrugations. As a result points of poor contact develop where the tips of the corrugations do not make perfect contact with the liner. It is therefore necessary to apply a sufficient amount of adhesive to the tips of the corrugations to fill in completely these areas of poor contact. Because of these adverse conditions, it has always been customary to heat the board during fabrication in order to cause the adhesive to set up as rapidly as possible. When the board passes from the double backer section of the corrugating machine to the cut-off knife, the adhesive bond must be sufficiently set up to hold the liners and corrugation together so that the board assembly can be cut into sheets and stacked.
>
> Until the advent of the "Stein Hall process," sodium silicate was the only adhesive of low cost that met these requirements to a satisfactory degree. Conventional starch or dextrin adhesives were not found to be suitable for this purpose because of their comparatively slow speed of setting. The reason for the superiority of sodium silicate adhesives over the conventional types of starch adhesives for this purpose lay in the fact that sodium silicate adhesives develop a very decided increase in viscosity with a small loss of their moisture content, whereas the increase in the viscosity of conventional starch or dextrin adhesives is relatively small, with a corresponding loss in moisture content

In the case of starch adhesives gelatinized *in situ* the increase in viscosity which results in the initial bonding effect is caused by the gelatinization *in situ* of the raw starch component of the adhesive when the board assembly is heated on the machine. It is apparent therefore that the time required to form the initial bond with the *"in situ"* type of adhesive is not dependent on the loss of moisture from the adhesive, but is dependent on the time required to heat the paper and adhesive assembly to the gelatinizing temperature of the suspended raw starch. Because of the great extent to which the viscosity of the adhesive can be increased by this means, it is possible to use from 3 to 5 parts of water to 1 part of solids in this type of adhesive and still obtain a sufficient increase in viscosity upon the application of heat to bond the paper satisfactorily when it comes off the machine. When applied to the paper, the *"in situ"* type of adhesive is in a free-flowing, relatively non-adhesive form but is subsequently converted, while between the paper plys, to an extremely viscous condition when the paper assembly is heated above 150°F.

The most commonly used adhesive of this type consists of ungelatinized starch suspended in an aqueous solution of a gelatinized starch product having sufficient viscosity to keep the raw starch in suspension and to cause the adhesive composition to be picked up and transferred properly on the applicator rolls of the machine. The gelatinized medium is referred to as "carrier." In addition to the carrier medium and the starch component of the adhesive, sodium hydroxide is usually included in the composition to lower the gelatinization temperature of the starch. In addition to sodium hydroxide, borax is also used to increase the degree of viscosity developed by the starch when it becomes gelatinized.

Unmodified starch or partially modified starch may be used as the ungelatinzed starch component provided it will form a viscous gel when cooked with 4 or more parts of water. It is this portion of the adhesive which when gelatinized *in situ* is responsible for the main bonding effect. This portion of the adhesive should be present in the proportion of at least 15% of the total solids of the composition if an effective result is to be obtained. It is general practice, however, to use a much greater proportion of unconverted starch than this specified minimum amount. Starches generally gelatinize at temperatures of 150°F. or more. Inasmuch as a gelatinization temperature of lower than 150°F. is desirable in order to obtain a more rapid formation of the bond, this normal gelatinization temperature of starch may be artificially lowered to the desired degree by the use of controlled amounts of a starch-gelatinizing agent such as sodium hydroxide. The carrier medium functions essentially to keep the raw starch component in suspension and to enable the composition to be picked up and transferred to the paper by means of the applicator rolls of the machine. It should be sufficiently dense nd viscous to keep the adhesive mixture from soaking too rapidly into the paper and et be fluid enough so that the adhesive may have the proper flow characteristics to be handled well in the adhesive pans and adhesive circulating systems.

Sodium hydroxide because of its effectiveness and low cost is the chemical generally sed as a means of lowering the gelatinization temperature of the raw starch component f the adhesive. The amount of sodium hydroxide used to obtain this result depends o a great extent on the proportions of the various other components of the adhesive and n the type of raw starch used. For high speed operation, it is desirable to operate with he gelatinization temperature as low as possible but not so low that premature gelatinization of the adhesive occurs in the paste pans of the machine. The general practice n corrugating is to operate with a gelatinization temperature of between 138° and 145°F. Borax is used in the adhesive formula, because it greatly increases the viscosity

> developed when the raw starch component is gelatinized. It also acts as an effective buffer for the sodium hydroxide.
>
> The preferred method of handling this type of adhesive is to use a continuous circulating system in which an excess of adhesive is pumped from the storage tank to the adhesive pans and the overflow pumped back to the storage tank. The temperature of the adhesive in the circulating system is thermostatically controlled to between 100 and 110°F. by means of hot water heating coils in the storage tank. This method of handling results in a uniform temperature of the adhesive regardless of weather conditions and avoids fluctuation of viscosity due to temperature changes. It also eliminates premature gelatinization of the adhesive in the adhesive pans. The proximity of the adhesive pans to the heated portions of the corrugating machine makes such premature gelatinization of the adhesive quite likely unless it is continuously circulated.
>
> The paste-mixing equipment used for preparing the adhesive in a corrugating plant usually consists of a primary mixing tank in which the carrier portion of the adhesive is prepared, a secondary tank in which the raw starch portion of the adhesive is dispersed and subsequently mixed with the carrier portion, and a storage tank for the finished adhesive.
>
> It is desirable that the paste be mixed for a total of 20 minutes or more after the carrier has been added to the secondary tank. This mixing breaks down the false body of the paste, so that by the end of this time it reaches its true viscosity and will have little tendency to further breakdown in the line. The gelatinization temperature of the paste should be occasionally checked and if necessary adjusted by using more or less sodium hydroxide. As a general rule the gelatinization temperature may be as low as 138°F. without producing serious gelatinization in the paste boxes, but if trouble occurs the gelatinization temperature may be raised by reducing the amount of sodium hydroxide. From the standpoint of speed of production, it is desirable to run with as low a gelatinization temperature as possible, with 138°F. being considered as the minimum safe temperature. The viscosity of the paste may be adjusted to suit plant conditions by varying the amount of carrier starch, and the paste should give a viscosity of about 27 seconds on a standardized pipette used for this purpose.

Originally corn starch was the starch most commonly used both as the carrier and as the main adhesive. Tapioca was used infrequently. During the war years when there was a scarcity of starch, a considerable amount of flour was used along with the starch. Generally this was wheat flour in which the properties of the protein were modified by rendering the protein as inert as possible. Flour, however, does not work as well as starch.

b. Starch As a Binder in Molded Products. Starch is used with mixtures of wood flour, pulp, clay, and other fillers (singly or in combination) as a binder in the manufacture of dolls, buttons, wooden heels, and other molded articles for which the use of synthetic resin is unsuitable or too costly.

In the "cold molding" process, the filler and starch are mixed together in a dough mixer or other appropriate mixing equipment and steam is injected into this mixture. The heat and condensed water gelatinize the starch, forming an intimate mixture of filler and binder; or the starch is first gelatinized with the water and the filler mixed into it to form a brittle "dough." In either case, the mixture is usually passed through a screen to break up lumps that may have formed, and is molded into the desired shape in steel molds under pressure. The molded articles are permitted to dry out slowly, preferably in chambers controlled for temperature and humidity. Controlled drying is necessary, for if the molded articles are dried too fast, the outer surfaces will harden and prevent the escape of water vapor from the interior. This will result in blow holes or, if the pressure is great enough, in cracks. On the other hand, if the drying is too slow, the compressed particles will tend to pull away from one another, resulting in a product which is crumbly.

Tapioca, because of the gummy characteristics of its gel, is most suitable as a binder. However, other starches can be used. The ratio of starch to filler will vary, depending on the type of filler and to some extent on the starch. With wood flour, a satisfactory ratio is 40% of starch and 60% of filler. The amount of water has to be adjusted so that the proper binding is obtained without oozing of moisture under pressure.

In the "hot molding" process, the filler and binder are mixed with water in the proper proportion, and the mixture is compressed in heated molds or extruded through heated tubes. The heat and pressure act simultaneously to gelatinize the starch and to mold it into the desired shapes. The drying is done in the usual way.

c. *Starch in Veneer Glues.* Starch is widely used as a veneer glue. However, while the starting point is raw starch, the finished glue represents starch which has become highly modified during the process of glue preparation. Unmodified starch requires a large amount of water to form a workable hydrosol. Such a glue contains comparatively little solids and, upon drying out, forms a thin glue film and a weak joint. Also, the large amount of water causes warping and operating difficulties. Therefore, it is necessary to modify the starch so that it is dispersed in a comparatively small amount of water. At the same time, it must be so modified that the finished glue has the proper viscosity. Should it be too heavy, it will not soak into the fibers and pores of the wood to form a good mechanical joint when pressure to the wood surfaces is applied. If too thin, the pressure when applied will cause the glue to penetrate into the wood too deeply and form what is known as a "starved" joint. The viscosity must also be adjusted so that the glue feeds properly from the rolls and the glue film transfers properly from one surface to another if only one surface is glued. Assembly, the type of process used (cold or hot), and conditions at the plant determine further necessary variations.

Tapioca is considered the best base for veneer glue. Ordinarily 2 to 2½ parts of water are used for every part of tapioca (by weight). If the agitation in the cooking kettle is good, the glue can be reduced further in viscosity through agitation, in which case the water can be reduced to as little as $1\frac{3}{4}$ parts. The tapioca and water are made into a slurry in the cooking kettle, and sodium hydroxide, dissolved in a small amount of water, is added slowly and gradually to the slurry while the latter is being agitated. The temperature is then raised and the mass heated at about 170°F. until the tapioca is thoroughly gelatinized. The amount of sodium hydroxide used is usually from 3 to 4% of the weight of the tapioca. Its effect is to produce a glue which is smoother and gummier than one made without it. It also undoubtedly acts on the wood cells to expand them and perhaps partially digest them so as to form a more intimate union between the wood surface and the glue. Sodium hydroxide, however, has the disadvantage that it tends to discolor certain woods. Care should therefore be taken in the proportion of the alkali used, particularly where the gumming has to be done on thin veneer. Chemicals other than sodium hydroxide, and oxidizing agents in particular, are also frequently used. These serve to modify the viscosity and properties of the glue still further.

Potato starch can be used in very much the same way as tapioca except that more vigorous agitation or longer agitation may be necessary in order to reduce the glue to the desired viscosity. With corn starch, larger amounts of water must be used. Generally, larger amounts of alkali are also used. This, of course, means longer drying time and greater chance of warping and staining.

Glues made from corn starch also have the tendency to set back on standing; that is, the hydrosol will upon standing be transformed into a hydrogel. Agitation will reverse the hydrogel into the hydrosol. Frequently a small percentage of sulfonated oil added to the adhesive will prevent this "pasting back." The use of resins, particularly of the urea formaldehyde type, to form water resistant veneer panels, has become quite widespread within recent years. These urea formaldehyde glues can be mixed with a considerable amount of starch or flour without affecting the water resistance of the finished panel. Here the amylaceous material acts mainly as a diluent.

d. Envelope Dextrins. Envelope dextrins represent the lower extreme of starch decomposition referred to previously. Here, the micellar aggregates of the starch have been highly disorganized and the molecular chains greatly shortened through scission and hydrolysis. The resultant products, which are "canary" dextrins, are highly soluble in water at room temperature, 95% or over, and exhibit little viscosity as compared with the original starch in equivalent concentrations.

The manufacture of envelope dextrins is discussed elsewhere. The chief properties to be sought for this type of dextrin through the process of dex trinization are high solubility, low viscosity, minimum reversion and low percentage of reducing sugars. While tapioca has always been found preferable as a base, recent studies have shown that waxy maize and sweet potato starch can also be used to prepare excellent envelope gums. It has also been possible to modify corn starch before dextrinization and to dextrinize such a starch into a satisfactory envelope gum. The results with waxy corn and with regular corn have been such that it is quite likely that these, either by themselves or in mixtures with tapioca, will now find a permanent place in the envelope gum field, even when supplies of tapioca are adequate. Waxy sorghum starch is similar in its characteristics to waxy maize.

Envelope dextrins are also known as seal gums, since they are used for the gumming of the flap or seal of envelopes. The requirements for a satisfactory seal gum are many and exacting. Chief among them are the following: In operation the gum must dry fast and must not "cotton" nor "sugar." It should form a finished seal which does not "block," nor curl, and which has the desired color, gloss, and smoothness. In use, the seal should absorb water quickly upon remoistening, to form a seal with the body of the envelope which does not separate from it upon drying. Fast drying is of extreme importance to the envelope manufacturer, for it regulates the speed at which the machine can be operated. The envelope machines most widely used are either the Plunger or the Rotary type, and these are generally operated at speeds of about 100 or more envelopes per minute. The drying time is therefore very short and within this short interval the seal must become dry enough not to retack when slight pressure is applied due to the accumulation of other envelopes or to packing. The fast drying is obtained by preparing a highly concentrated dextrin dispersion and by watching the amount of sugar in the dextrins or plasticizing agent added.

"Cottoning" and "sugaring" are two extremely troublesome factors often experienced with the Plunger type of machine. "Cottoning" is the term applied to fibers of gum formed by the "pickers" traveling from the gumming roll to the paper. The pickers, which pick the gum from the gumming roll and stamp it upon the paper, travel at a great speed, and unless the gum breaks clean from the pickers, fibers will be formed. These fibers accumulate and necessitate stopping the machine from time to time to clean them off; otherwise they may drop on the paper or envelopes. As might be expected, cottoning is particularly pronounced on dry days, since the lower humidity causes faster drying out of these strands. It may be prevented or minimized by the incorporation of substances that will make the gum "discontinuous." Thus, the incorporation of a small amount of starch will stop cottoning. Unfortunately, it will also dull the seal.

"Sugaring" is the opposite of cottoning. It is due to the gum being "short," and instead of fibers being pulled out, particles are thrown off the gumming roll on the envelopes.

The finished seal should have good gloss. This is usually the case when the dextrin is properly made, though only tapioca gives a really high gloss seal. Some envelope manufacturers prefer darker seals than others. The color can generally be adjusted during the dextrinization and also by the use of coloring agents. The seal should be smooth, or the remoistening may be uneven. A mottled seal is not necessarily the fault of the gum. It may be due to the paper, if the latter has a finish that is not easily wetted by an aqueous adhesive. However, if the gum lacks good flow, mottling will result.

The period between the manufacture and the use of an envelope may be as long as 2 years, and it is important that the envelope neither seal itself, "block," owing to the high humidity at one period or another during storage, nor that the seal curl. An envelope seal may curl owing to extreme brittleness of the gum. The type of paper used may accentuate "curl" still further. If the gum is brittle and the paper is non-absorptive, the film is entirely on the surface of the paper. This creates a strain between the two surfaces of the paper and causes excessive curling. If the gum is absorbed into the paper, this strain is somewhat relieved and curling is therefore less. Generally, curling is eliminated and flat seals are produced by the addition of plasticizers, such as glycerol, ethylene glycol, invert sugar, sorbitol, sorbitol esters, etc. The amount of plasticizer added to the gum must be carefully regulated, or these hygroscopic plasticizers will cause blocking. If glycerol or diethylene glycol is used, about 2% based on the weight of the dry gum is the amount generally required.

Seal gums are composed of dextrins which are highly dispersible in cold water. Nevertheless, the characteristics of the gum and of the seal, such as adhesion and appearance, are greatly improved by preparing the gum with heat so that no undispersed dextrin is present in the finished gum. The general practice is to mix the gum with water in the concentration of about 65% solids for the Plunger type of machine, and in somewhat lower concentration for the Rotary type machine, and heat, with agitation for about 45 minutes at 180°F. The dispersion is then drawn off and ready for use. It is important that the prepared gum maintain its viscosity over a reasonable period of time, for the manufacturer does not always use it up within a few days of its preparation. Should the consistency of the gum change during storage, the working characteristics of the product are impaired. For instance, should the gum thicken, the operator will add water in order to reduce the viscosity to the proper working consistency. This, however, will change the percentage of solids, and form a thin seal with poor adhesion.

Canary dextrins of the seal gum type have been discussed in detail because, as mentioned before, they represent starch degraded to the maximum degree for adhesive purposes. These dextrins can be and are used for a number of other adhesive purposes besides envelopes, particularly when quick tack and fast drying are required.

They have, however, certain drawbacks which make them unsuitable for a great variety of other adhesive uses. Chief among their disadvantages is the ease with which these dextrins absorb moisture. Owing to the extreme dextrinization treatment, the dextrin unit is small and contains many exposed hydrating groups which will associate with water vapor in the air. This swells the film and weakens the bond between the two surfaces, particularly when the bond must withstand strain, as in the case of paper bags. Another disadvantage is the extreme brittleness of the gum film. Still another is the fact that these dextrins will function properly only when made up in highly concentrated solutions, which means that their spread, in terms of solids per unit area of surface, is low and the gumming costly. As compared with these envelope dextrins, which take only 1 part of water to 2 parts of solids, adhesives can be prepared from less degenerated canary dextrins, and from white dextrins, or British gums, that will take anywhere from 1 to 5 or more parts of water per part of solids. These variations can be brought about by gentler treatment of the starch micelle, so as to avoid excessive degradation, and by the addition of chemical substances that affect the colloidal characteristics of the micellar fragments and their peptization by water. They constitute large classes of adhesives and have the advantage of lower cost, greater "deformability," and varying degrees of resistance to reabsorption of water vapor.

e. Envelope Seam Gums. The adhesive used for the sealing of the back seam of the envelope must meet a different set of specifications from that of the seal gum. The drying need not be so fast. As a matter of fact, it is preferable that the gum dry slowly so that no strains are developed in the paper, which might cause loss of shape of the envelope. It must be highly plasticized so that the envelope seam will be flat. It must not soften the paper unduly and cause it to pucker, nor must it cause discoloration of the seam on aging. Above all, the seam must be well stuck.

To meet these specifications, the gum is made from white rather than canary dextrin as the base and is combined with plasticizers. The plasticizer assures a flat seam and slow drying, while the white dextrin gives a strong bond, light color, and also contributes to the slow drying and improving flexibility of the adhesive. The plasticizers used are generally sugar or urea and may represent from 30 to 50% of the weight of the dry adhesive.

The sugar-plasticized gum is the type that has been most generally used until recently. It contains up to 50% of sucrose and is prepared by heating the dextrin-sugar mixture in an acetic acid solution of about 35% concentration. The heating is done at 180°F. for about 30 minutes. The acetic acid inverts the sucrose, forming invert sugar, which plasticizes the adhesive through its hygroscopic properties. This plasticizing effect is further enhanced by the hygroscopicity of acetic acid itself. For Plunger machines the gum is prepared with about 70% of solids, 20% of acetic (56% solution), and 10% of water. For Rotaries, more water can be used. As an adhesive, this type of seam gum is particularly effective, for the acetic acid also attacks the paper fiber somewhat and forms a better bond, particularly when the papers are hard sized. Its chief disadvantages are the odor of the acetic acid, to which the workmen in the envelope manufacturing plants object, and the tendency of the invert sugar to brown on aging and form a discolored seam.

The urea type of envelope seam gum (18) is made with urea as the plasticizing agent. It forms an excellent adhesive and in addition is free from the disadvantages of the sugar gum both as to odor and discoloration. In preparing the gum the heating is done at temperatures lower than those for the sugar gum. Temperatures between 140 and 150°F. are preferred. Higher temperatures start decomposition of the urea and the evolution of copious volumes of gas. The prepared gum contains about 77% solids when intended for use with the Plunger type machine, and for the Rotaries the water is generally increased to about 30%.

The back seam gums, and particularly the acetic acid type, are often used as the adhesive for sealing in the windows of envelopes.

Seam gums, like seal gums, must remain fluid when once prepared and must maintain a fairly uniform viscosity over a reasonable period of time. In some cases, envelope manufacturers buy their gums in the liquid form. Such solutions, of course, must maintain their fluidity and uniform viscosity over a period of months.

f. Bag Gums. In the manufacture of bags, several types of adhesives must be used. If the bag is single ply, such as grocery bags, sacks, etc., a seam gum is needed to form the seam along the side of the bag, and a bottom gum to seal the folds which make up the bottom of the bag. For a bag of several plies, a ply paste is also necessary to seal the plies together at the seam and at the bottom. Often the seam gum can be used for pasting the plies and for the seams. For forming the bottom, however, a more viscous gum is generally used. For the seams and plies, the adhesive must act considerably faster than for the bottoms, and consequently the seam gums generally contain less water than the bottom gums.

Although bag gums could be prepared with starch as the base and modified with chemicals during the heating, most of these gums are made from white dextrins or British gums. The type of dextrin or British gum used will determine the amount of water needed to prepare a gum of proper working viscosity, tack, drying time, bond, and deformability, and these factors are further modified by the addition of chemicals, such as borax, sodium carbonate, sodium hydroxide, etc.

The chief characteristics necessary for a good bag gum are the proper consistency, so that in operation at high speed it neither "throws" or spatters, nor "strings;" it must not discolor the paper; must possess the right tack for the particular speed of operation; and must, of course, form a strong bond, *i.e.,* one that will pull fiber if an attempt is made to tear apart the joined surfaces after they have dried. The selection of an adhesive to meet these characteristics will depend principally on the paper, the type of machine used, and the speed of operation. Thus, for a Potdevin machine, which makes the complete bag in one operation, a seam gum is necessary which will set fast, so as to prevent the seam from moving or slipping under the strain when the bottom is formed a few seconds after the seam is glued. With Smith and Winchester and with Coty machines, on which the seams are made and the bags are stacked for subsequent sealing of the bottoms in a different operation, the seam gum need not set so fast, as the seams have more time in which to form the necessary strong bond. Newer types of these machines are now available which glue seams and bottoms in one operation. This, of course, necessitates faster setting glues.

The paper used is an important factor in determining whether a given adhesive will function satisfactorily. A soft paper is simpler to stick than a hard sized paper, but in any case, the size should be of the type which permits the paper to be wetted by aqueous solutions. If such is not the case, chemicals must be incorporated into the adhesive which will dissolve the size or change the surface so that wetting will take place. As an illustration, carbon tetrachloride will help in the case of wax-coated paper, and sodium hydroxide in the case of rosin-sized sheets. The rôle of chemical substances in adhesives will be discussed in greater detail later in this chapter. However, there are some sizings which remain unaffected by the chemicals that can be used in aqueous adhesives. A great deal of trouble has been experienced recently with the high-wet strength type of paper when resins have been used for increasing the wet-strength.

Since there are many types of dextrins and British gums, and these in turn can be greatly modified in their characteristics by the addition of chemicals, it is obvious that a large number of bag adhesives is possible. These may vary in water-absorbing characteristics from 1 part of gum to 1 part of water, to 1 part of gum to 5 parts of water, and may or may not require heat for preparation. When heat is required, the heating is generally done at 180°F. for about 15 minutes, with agitation. Most bag gums are used cold. Sometimes, when quick setting is essential, it is advantageous to use the gum at temperatures from 120-140°F.

Recently a wide demand has arisen for gums that will form water-resistant seams and bottoms. These will be discussed later under water-resistant adhesives.

g. Tube-Winding Gums. Starch adhesives are used widely in the manufacture of paper tubes, paper cans, paper cores, etc. In these operations the paper travels from a roll, is gummed by an applicator roll from the gum box, and the gummed paper is wound on a shaped mandrel into a tube, can, core, etc. The wound product leaves the mandrel and is cut to size after it has traveled a certain distance from the mandrel. In the case of tubes and cans, the bottoms are sealed in a subsequent operation.

The number of plies of the formed article will depend on the number of turns of the mandrel. Sometimes different papers are used for the different plies in order to produce a tube or can that meets special requirements such as grease-proofing water resistance, etc. The adhesive used for these special papers is usually different, and in such cases special paper has its own gum box.

There is "spiral" winding and "straight" winding, and there are several different types of machines for each type of winding. These, as well as the paper used and speeds of operation, have an important bearing on the choice of a satisfactory adhesive for a particular tube-winding operation. In all cases, one of the most important factors to be considered in selecting the right adhesive is the distance the tube travels before it is cut off, for it is essential that at the cut-off the plies of the paper have stuck together well so that they will not separate at the edges, forming "dog ears." If the distance is short, say 1 ft. or so, the adhesive used must set very quickly; therefore the adhesive must be one prepared with a base that is highly dispersible in water, such as one of the canary type of dextrins, and the amount of water should be kept down to the minimum necessary for the proper viscosity for efficient operation. About 1 part of gum to 1 part of water is the usual ratio. Often the properties of the adhesives are further modified by the addition of alkalies, which serve to increase the speed of the tack, to get a better "bite" into the paper if the paper has a hard finish, and to permit the use of somewhat larger amounts of water, thus increasing the spread and reducing the cost.

As the distance between the mandrel and knife increases, the time given for the adhesive to set is longer. This means that the adhesive may contain more water and still form a satisfactory bond between the plies by the time it reaches the cut-off. In such cases, starch decomposition products of lower solubility can be used, as they permit the use of more water in the preparation of the liquid adhesive and result in greater economy in the cost of the glue per unit area of paper. The amount of water a particular operation will tolerate does, of course, vary and the adhesive may be selected so as to take 5 parts of water or even more per part of solids when the cut-off is very long, 60 ft. or more, and less water as the distance to the cut-off grows shorter.

Tube-winding gums, if they are not purchased by the consumer in the liquid state, may be of the "cold water-soluble" type or may require heat for preparation. With the former, all that is necessary is thorough agitation with water; with the latter type, the heating is best done at 180°F. for about 15 to 30 minutes. These may be used cold or warm as in the case of bag gums. Maintenance of uniform viscosity of the prepared gum over a reasonable period of time is also important.

h. Gummed Paper. The adhesives used for "flat gumming" or for gumming paper which is used for labels, stamps, stickers, etc. are generally of the same variety as those used for envelope seal gums. The requirements for such a gum are very much the same as those enumerated for a good seal gum. The brittleness of the gummed film, which has to be counteracted in an envelope gum, is often an advantage for gummed tape, since in many cases the gummed sheets are run over a "breaker bar" which breaks the gummed film into numerous small fractures. Thereby the uneven strain between the gummed and ungummed surface of the paper is reduced, and the sheet lies flat and has little tendency to curl.

The working viscosity of the gum is generally somewhat lower than that for envelope seals, and the concentration is usually about 50% of solids. The gum, prepared by heating with water at 180°F., is generally applied by applicator rolls from the gum box to the paper and is usually dried by passage through heated tunnels.

i. Paper Tape. Until recently, the gum used on the face of paper tape has been universally of the gelatin variety. The requirements for gummed tape are very severe. It must absorb water evenly and develop even tack, or else the tape will stick only in spots. It must, when moistened, have a certain amount of "slip" to enable the user to adjust it slightly if it has not been placed in the correct position. Its initial tack must be fast, but the gum must set slowly so that its tack is not lost if the operator is slow in using the moistening tape. In other words, it must have good "durability." It must not curl, and last but not least, it must form a good bond with the surface to which it is applied.

Within the past several years, a vegetable gum has been perfected which meets these requirements (19). The success of this development is based principally on the combination of urea with starch decomposition products that are not too highly degraded and, therefore, form stronger bonds and less brittle films than would be the case with canary dextrins. Ordinarily such decomposition products would require more water than could be tolerated in the manufacture of gummed tape. However, through the use of urea, the amount of water is decreased, and dispersions can be prepared which are concentrated enough (about 50%) to impart the necessary working characteristics for the manufacture of gummed tape and still permit the desired speed of drying so as not to reduce output.

Through the use of these less disorganized starch products in combination with urea and other modifying agents a tape is produced which meets the exacting requirements mentioned before. Its freedom from odor is an additional advantage. In cases in which unusual quick tack is required, it has been suggested that it be moistened with a solution of borax rather than with water (20).

The adhesives used for gummed tape are prepared by heating with water at between 180 and 190°F. for 15 to 30 minutes. The application is very much the same as that for flat gumming, and the drying too is generally done in heated tunnels.

In the "combined" tape, that is in paper tape which is combined with a cloth backing, the combining adhesive used is generally a dextrin product. The specific requirement is a good bond, but the adhesive must be one that is highly plasticized so as not to make the tape too stiff and brittle; *i.e.*, it must have the proper Elmendorf test value. Urea adhesives have been found quite satisfactory.

The combination of cloth and paper for combined tape may also be accomplished by the use of starch through gelatinization *in situ* (21).

j. Laminating Adhesives. Starch adhesives used for laminating paper or board will vary widely in their composition, depending on the paper or board to be laminated, on the type of laminating machine, the speed of operation, the temperatures, etc. In some cases, dispersions of starch itself may be satisfactory. The laminating adhesive in such a case contains a considerable amount of water, as no working gel can be made from starch in concentrations which are much greater than about 12% of solids. Such a dispersion can be used only when the operating speed is low and the time for drying is long. On the other hand, there are some types of lamination, such as mounting printed paper on a paper board backing, for which the adhesive used should contain comparatively little water, 60% or less. In these types of laminating adhesives, it is not only the speed of tack that determines the type of adhesive, but also the necessity of minimizing warping due to strain between the two types of paper. Generally, with less water there is less warping. In many cases, plasticizing materials are incorporated with the adhesive, particularly when quick tack is not essential.

Between the two extremes starch on the one hand and a highly degraded dextrin which can be prepared with comparatively little water on the other hand, lies an entire range of adhesives used for laminating purposes. These vary in the percentage of water needed, in the viscosity, tack, etc., and are dependent on the conditions under which the particular adhesive has to function. As an illustration, for a Parry liner, a machine which is very widely used for laminating purposes, a product which takes 3 parts of water is generally found quite acceptable.

Laminating adhesives may be "cold water-soluble" or may be of the type that requires heat for dispersion. The methods for the preparation of the liquid adhesive from the dry product are the same as discussed previously. Lamination may also be effected by "gelatinization *in situ*" (21) of starch in a manner similar to that described in the earlier discussion.

k. Case-Sealing, Carton-Sealing, and Labeling Gums. The adhesives used for sealing cases, cartons, etc., may contain as a base a white dextrin, canary dextrin, or British gum, the particular base used being dependent on the function of the adhesive. In the compounding of the adhesive, the bases are further modified by the addition of chemicals. When the sealing is done on an automatic machine with a belt conveyer, the adhesive must set very fast if the freshly sealed case or carton travels only a short distance under the pressure of the belt. In such cases, a canary dextrin of high solubility, one that will take comparatively little water, is the one to be used. A suitable adhesive is one that takes about 1 to 1½ parts of water for each part of gum; when the sealing is done by hand or when the carton travels under the belt pressure for a considerable distance, the amount of water may run as high as 4 parts or more for each part, by weight, of the gum. Sometimes, when porous board has to be sealed, it may be advisable to use a fairly dilute case-sealing gum and to thicken it with an inert filler, such as clay, that prevents the adhesive from soaking into the board too freely.

l. Cold Water-Soluble Gums, Box Gums. The term "cold water-soluble" gum is applied to dry adhesives which do not require heat for dispersion. These adhesives are prepared simply by mixing with water and agitating the mixture. In use it is found advisable to let such dispersions age for 24 hours or so in order to get maximum efficiency in operation. This, of course, is due to a more complete dispersion and to a stabilized colloidal complex.

As would be expected, the bases for cold water-soluble adhesives are canary dextrins and British gums which are highly dispersible in water at ordinary temperatures. The manufacture of these bases requires considerable skill and the proper type of raw material. High quality tapioca flour is the best. At best, however, complete dispersibility of the dextrin or British gum is difficult to obtain. In practice, these bases are mixed with alkaline chemicals, which assist in the dispersibility and also help modify the adhesive characteristics and the water-absorbing capacity of the finished dispersion.

Cold water-soluble adhesives can be used for most adhesive purposes, bag gums, tube winding, lamitation, etc. Their widest use, however, is in the manufacture of cardboard boxes, for which purposes they are employed as adhesives for applying the outer paper wrap to the cardboard. There are two standard methods for this operation, "stripping" and "tightwrapping," and the adhesives necessary for each are different in their requirements.

"Stripping" is the term used to designate the gluing of the paper wraps to the board by hand or slow machine operation. The speed of the tack is comparatively slow and therefore adhesives can be used that are low in solids, 20 to 25%. "Tightwrapping," however, is a fast machine process, and the rate of tack must be quick. For this purpose an adhesive must be used which contains comparatively little water and which has rather critical characteristics. The Stokes and Smith machine is almost universally used for the "tightwrap" operation. The paper wraps, cut to the proper size for the particular box being manufactured, are gummed and conveyed on a belt conveyer to the operator at the machine. The operator places the gummed wrap in position over the cardboard form, and the gluing is done by the Stokes and Smith machine at a fast rate. The adhesive must be of the proper viscosity so that no trouble is encountered in the gumming of the wraps or in producing a thin film. It must not be too slow in tack or the paper edges turned over the cardboard form will spring back when the pressure of the machine is released. When this is the case, the operation must be slowed down by placing fewer gummed wraps on the conveyer so that each wrap has a chance to "temper" longer before it reaches the operator. At the same time the adhesive must not set too fast or there will not be sufficient tack left by the time the wrap reaches the end of the belt where the operator transfers the wrap onto the form. It may also cause unadhered spots or "blisters." The amount of water in the gum is of importance not only to increase the speed of tack but also to minimize warping. Generally adhesives for tightwrap papers contain from 40 to 50% of solids.

Of primary importance in selecting the proper adhesive for a particular operation is the nature of the paper and board used. The porosity, size, coating, etc., all have an important bearing on the choice of the proper adhesive.

There are numerous other uses for adhesives besides those enumerated above. To discusss them all is beyond the scope of this chapter.

m. Water-Resistant Starch Adhesives. Recently a wide demand has arisen for starch adhesives which, upon drying, will become water-resistant. Although there has always been some use for such a product, the need for it has become of increased importance owing to the government requirements for fiber cases or cartons that will be water-resistant and that can be used for shipment of goods overseas to the armed forces.

The best results in the development of such an adhesive have been obtained from the combination of a starch base with urea-formaldehyde resins. The combination is not water-proof or water-repellent, as the bonding film will absorb moisture even after it is thoroughly dried, but if the adhesive is properly compounded, the absorbed misture will not seriously weaken the bond or cause adhered surfaces to fall apart or delaminate, even under considerable strain.

One of the most important points in forming a water-resistant adhesive with a urea-formaldehyde resin is the selection of the proper adhesive base. The underlying principle for guiding this selection is that the base be as little disorganized as possible. The fewer the free hydrating groups present in the starch product and the fewer the number of primary valences, the less is the quantity of resin required to develop resistance to water. The free hydroxyl or hydrating groups in the degenerated starch micelles are undoubtedly the points of entry from which the micellar aggregates or fragments go into dispersion when mixed with water. It is fairly certain that the urea-formaldehyde polymer combines with the available free groups to block these sorption points. Therefore, the fewer free groups present, the less resin is required to block them. Thus it follows that the less disorganized or fragmented the starch decomposition product used as the adhesive base, the less is the quantity of resin required to produce a finished adhesive which will, upon drying, become water-resistant. In practice it has been found that in manufacturing water-resistant laminated board with raw starch and the method of gelatinization *in situ* (21), smaller amounts of urea-formaldehyde resin can be used to procure products equivalent in water resistance than are required when starch decomposition products are employed. British gums and oxidized starches should be more satisfactory than dextrins of equivalent viscosities, and this too has been confirmed in practice.

The urea-formaldehyde polymer can be of varying degrees of polymerization as long as it is in the "water-soluble" state at the time of its use, although the degree of polymerization has an important bearing on its subsequent reaction with the starch product and the speed at which water resistance is developed (14). Generally in order to develop water resistance fairly soon after the adhesive is applied, a catalyst is used. The catalyst in such cases is the hydrogen ion. The greater the concentration of the hydrogen ion, or the lower the pH (within certain limits), the faster will polymerization take place, with the simultaneous development of water resistance. Unfortunately, the faster the resin polymerizes, the shorter is the life of the adhesive. As the resin-adhesive combination polymerizes, it tends to become more viscous and pasty and loses the fluidity necessary for its satisfactory application. The rate of the development of water resistance must therefore be balanced against the rate of increase of viscosity of the adhesive, and a satisfactory compromise selected. In practice, it has been found that a pH of about 4.5 to 5.5 will permit the paste to maintain its proper fluidity for a period varying from 8 to 24 hours, depending on the temperature of operation, the concentration of the resin, and the type of adhesive base used. The proper pH is generally obtained through the use of an acid salt, such as ammonium chloride, aluminum sulfate, etc. The amount of catalyst used is generally 10% of the weight of the resin, although this will vary depending on the type of resin. When the percentage of the resin used is small, it is desirable to increase the percentage of the catalyst up to 25% in order to get the maximum water resistance from the resin-adhesive combination.

Excellent application of the water-resistant adhesive is being made now in the manufacture of corrugated and solid fiber board used for making corrugated and fiber cases for shipment abroad. Specifications on these types of board are JAN-P-108 (22).

These specifications are met by the combinations of starch with urea-formaldehyde in the method of gelatinization *in situ*, or by the combination of a starch degeneration product with urea-formaldehyde in the usual procedure of preparing the adhesive with water by heating. Formulae typical of both of these applications are as follows:

(*a*) Gelatinization *in situ*: 234 lbs. of carrier are heated at 180°F. in 84 gals. of water, cooled to 140°F., and 126 gals. of water are added. 468 lbs. of starch are then added, and the mix agitated to form a uniform dispersion. The pH is adjusted, if necessary, to between 7 and 8 with sodium carbonate. When the temperature of the mix is about 90°F., the urea-formaldehyde resin is added. After the resin is added and before this mix is used, the pH is adjusted to about 5.5 with an acid salt.

(*b*) Dextrinized starch is heated with water at 180°F. in a concentration of about 35% of solids. When thoroughly peptized, the pH is adjusted, if necessary, to between 7 and 8, the resin added, and the pH again adjusted to between 4.5 and 5.5 before use.

3. **Manufacture of Adhesives.** In the manufacture of adhesives, the important points to consider are the type of base, the method of manufacture, and the effect of added chemical agents on the properties of the adhesive.

a. Type of Base. Theoretically, any starch can be used as raw material for the manufacture of the starch derivative to be used as an adhesive base. The types of bases obtained from the different starches are, however, widely different. The starches of commercial importance are, principally, corn, potato, tapioca, and to a lesser degree, wheat, sago, and sweet potato. The structure of the different starches, the differences in chain lengths, straight and branched chains, etc., as well as the structural reasons for the different properties of the different starches are discussed elsewhere. For our purpose here, we need to point out only that tapioca, waxy maize and sweet potato starch represent* the best material for the manufacture of adhesives.

The chief reasons for the superiority of these starches over starches of other origin are that (*a*) their dispersions are clear, (*b*) the dispersions maintain a more uniform viscosity over a period of time as compared with dispersion of other starch bases, probably owing to the lesser tendency of these starches to revert as compared with other starches, and (*c*) these starch adhesives are, as a rule, gummier than adhesives made from other starches.

Corn starch, which commercially is next in importance to tapioca flour for the manufacture of adhesives, forms products that are opaque and films which are rather dull in appearance. Aqueous adhesives made with corn starch bases have a tendency to form thixotropic gels; that is, the adhesive will, upon standing, change from a hydrosol to a hydrogel which, upon agitation, reverts to a hydrosol (becomes fluid again). This is repeated any time the adhesive is allowed to stand unagitated for several days. The manufacturer using adhesives also finds that with tapioca products he has less trouble in maintaining production because of the lesser tendency toward changes in viscosity. Also, the adhesiveness of corn hydrosols is not as good as that of tapioca. Their consistency is "short" as compared with the "long," gummy consistency of tapioca. Besides, corn starch adhesives generally form more brittle films than tapioca adhesives, particularly when white dextrins are used as the base. In spite of these differences, corn starch is used as a base for many adhesive products when quick tack and high speed of operation are not of paramount importance.

---

*Little if any sweet potato starch is now being manufactured due to the failure of the U. S. Sugar project at Clewiston, Florida. Waxy sorghum, however, might assume increased importance.

Potato starch forms adhesive bases which are satisfactory for many purposes. The adhesiveness of many potato starch bases is nearly as good as that of tapioca flour bases, and the films are clear and glossy. However, potato starch adhesives "set back" (revert) faster than tapioca adhesives. This constitutes a great disadvantage, for, while, they can be used satisfactorily at elevated temperatures, it is difficult to use them at room temperature, particularly when the percentage of solids is high. Another, although minor disadvantage of potato starch adhesives is their odor and taste; potato dextrins have a peculiar odor and taste, sometimes described as that of a cucumber, which is carried over into the adhesives. A further drawback is the high price of potato as compared with other starches.

Adhesive bases obtained from wheat starches are similar in the main to those made from corn starch. As compared to corn starch, they have one objectionable feature; that is, foaming. Wheat starch adhesives foam excessively, and even with the use of foam depressants it is not always possible to eliminate the foaming entirely.

The work done with sweet potato starch indicates that it forms excellent adhesives with properties closer to those made from tapioca than to those from corn, wheat, or potato starches. Owing to the comparatively small amount of sweet potato starch available, its use for adhesives has been limited.

Adhesive bases made from starches are usually in the form of canary dextrins (of high solubility), white dextrins (of low solubility), and British gums (of high or low solubility). To a lesser extent they may consist of modified starches, such as thin boiling, oxidized, or enzyme-modified starches.

b. Methods of Manufacture. The principal method for the manufacture of adhesives consists of blending the proper dextrin or British gum bases, obtained by the usual method of roasting starch, with the necessary chemical substances to obtain a final product that will possess the characteristics essential for the particular purpose for which the adhesive is intended. Generally, the user of these adhesives will himself perform the final step in their manufacture; that is, the preparation of the liquid adhesive from the dry by the addition of water and by heating. If the dry adhesive is of the "cold-soluble" type, no heating is needed. In many cases, this final step is also performed by the adhesive manufacturer, so that the consumer buys liquid adhesives which are ready for use as received, with the possible exception of further dilution with water to adjust them to the proper working viscosity.

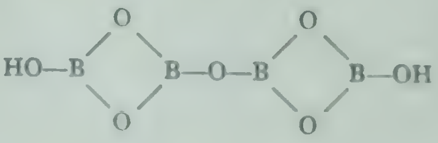
It is possible in some cases to use unmodified starch as a starting point and modify it during the heating period in water by the addition of chemicals (23). The preparation of veneer glue is an example. However, the controls necessary for preparing good adhesives in this manner are exacting and are difficult to establish in the majority of plants. The manufacturers of liquid adhesives seldom use this method, as it does not lend itself to the manufacture of products of uniformly high quality. Most of the liquid adhesives made by adhesive manufacturers when starch, and not dextrin or British gum, is the starting point, are produced by means of enzymes or acids. With enzymes, the α- or liquefying amylases are used as free as possible from the β- or saccharifying amylases so as to form as little sugar as possible, since the presence of an appreciable amount of sugar is undesirable for most types of adhesives. When enzymes are used with starch to produce adhesives, the starch slurry is mixed with the required amount of enzyme and, after the pH of the slurry has been adjusted to the optimum point for conversion, it is heated at the proper temperature until the desired viscosity is obtained. In determining the best temperature for a particular conversion, consideration should be given to the kind of starch used. The minimum temperature for the conversion must be slightly above the gelatinizing temperature of the particular starch, and differs for the different starches. However, the enzymes must also be selected with care, for the commercial enzymes on the market vary and have their own optimal conditions for best performance, particularly as to pH, temperature, and concentration. In all cases, the enzyme must be inactivated after the desired viscosity is obtained, so that the finished product does not continue to thin on standing. This inactivation may be done with heat, or by chemical means through the use of enzyme inhibitors.

In the preparation of concentrated adhesives from starch, it may be necessary to carry out the conversion in several stages, particularly for corn or wheat starch. In such cases, a conversion may begin at a concentration of 15 to 20% of starch solids and be allowed to proceed as far as it will go. More starch and enzyme are then added and the conversion continued. This may be repeated until the required amount of solids is obtained. For an adhesive requiring quick adhesion and quick drying the percentage of moisture cannot be much above 60%. In many cases, it is impossible to start out with a 40% starch slurry, and it is therefore necessary to resort to conversion by steps. As is the case with dextrins, or British gums, chemical substances are added to these liquid conversion products to make the finished product. The kind and amount of chemical agents depend on the specifications for the particular adhesive.

There are a number of dry, "cold water-soluble" adhesives, which are known as "drum dried" or "hot roll" products. These are made by heating the proper dextrin, British gum, or modified starch with water, adding the chemicals necessary for the particular adhesive, and then removing the water by evaporation on a revolving heated drum. Instead of a modified or dextrinized starch, raw starch may be used as the starting point and modification brought about by means of enzymes, or a combination of an enzyme-converted material and a dextrin material can be used. The dry product made from the proper base and containing the requisite chemical additions comes off the roll in the form of flakes and then is ground to the proper particle size for the finished product. One of the important points of superiority of these hot roll products over other "cold water-soluble" products is the ease with which they disperse in water without lumping.

c. Chemical Additions. Borax is the most important single substance among the many used to modify adhesives. The man who first discovered the effect of borax on starch adhesives has made the most outstanding contribution in this field. At the time this discovery was made, the reasons for the effect were obscure. Today we understand these better, though we are still uncertain which of the possible mechanisms plays the most important rôle.

Compounds containing free hydroxyl groups will in aqueous solution combine with some of the water, through hydrogen bonding, to form complex molecules. The water that has entered into chemical combination with the dissolved or dispersed substance is "bound," as distinguished from that portion of the water which is not chemically combined and which thus remains "free" and acts as the solvent. The removal of water from the solvent changes the characteristics of the solution, and particularly the viscosity. When starches or dextrins are dispersed in water, some of the water is chemically bound with the starch or dextrin through the exposed hydroxyl groups, while a portion of it remains as free water. Borax, which is considered the sodium of salt of dihydrotetraboric acid, will on solution with water form a certain amount of free acid. The structural formula (*24*) of the acid may be regarded as



When borax is added to a starch or dextrin dispersion, the resulting effects may be due to the association of the borax or dihydrotetraboric acid molecule with some of the remaining free water, to the coupling of the borax molecules (hydrated or non-hydrated) with the dextrin or starch molecules* (hydrated or non-hydrated) through hydrogen bonding, or to both. This results in new combinations having properties that differ in one or more respects from the original mixture. As the number of exposed hydroxyl groups in starch and dextrin may vary greatly, it is obvious that the possible combinations are many.

To the chemist dealing with adhesives, the most important effect of the addition of borax to dextrin or British gum is the resulting increase in viscosity and the "gummier" characteristics. It is possible to take a British gum of high solubility which, by itself, will form a workable hydrosol only in high concentration (*e.g.*, 50% of solids) and, through the addition of borax, form an adhesive which contains only 20% of solids and is equivalent in viscosity and tack to the more concentrated hydrosol. Adhesives treated with borax tack faster, as a rule, than adhesives not containing borax. The bond between the two surfaces to be held together is quicker, thus permitting greater speed of operation by the manufacturer. The reason for the faster tack is probably the comparatively small amount of free or solvent water present in these borated adhesives. As the amount of solvent is small, the dispersed material is nearer the saturation point and is thus very sensitive to further slight changes in concentration. In the use of the adhesive, the loss of a slight amount of solvent water, through absorption or evaporation, is sufficient to transform the hydrosol into a hydrgel. The effect of borax on the adhesive bases varies widely with the type of base used. Little borax is necessary to increase greatly the viscosity of a starch dispersion, although with dispersions of modified starches, dextrins, or British gums, greater amounts of borax are needed.

In the formation of adhesives with dextrins or British gum, the viscosity and tack are increased with increased concentration of borax, up to a certain point. If the amount of borax used is excessive, the resulting dispersion cannot be spread into a smooth continuous film. Apparently too much free water is removed, not leaving enough solvent water to give the liquid the necessary fluidity. The adhesive also loses its tack and becomes rubbery. A partial explanation for this loss of tack is, probably, the lack of free hydroxyl groups necessary for wetting the surfaces and for specific adhesion.

Most adhesives, with the exception of those that are prepared at an extremely high concentration of solids, such as envelope adhesives, contain borax. Borax is essential for the preparation of "cold water-soluble" starch products and for this purpose it is often used in combination with other alkalies. The use of other alkalies with borax modifies to a certain extent the effect of the borax. These alkalies modify the characteristics of the finished adhesive further. Chief among such alkalies are sodium hydroxide and sodium carbonate. Sodium hydroxide cannot be used in dry blends, but it is widely used by the manufacturers of liquid adhesives or by the users of adhesives in order to overcome difficulties that may have arisen in connection with a particular adhesive. When used with an adhesive containing borax, the effect of sodium hydroxide is to increase the viscosity and the rate of development of tack still further. This effect is particularly noticeable for small amounts, *e.g.* 0.2 to 0.5%. Amounts larger than 1% are inadvisable. The effect of sodium carbonate is due principally to the sodium hydroxide formed through hydrolysis. Adhesive bases not containing borax are not appreciably affected in viscosity by the addition of sodium hydroxide or sodium carbonate. If too much of these alkalies is used without borax, thinning of the adhesive dispersion rather than thickening will take place.

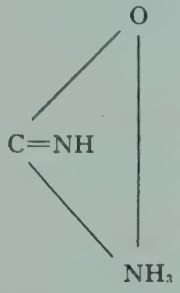
---

*Borax materially retards the association and spontaneous precipitation of the linear polymer fraction of starch. Ed.

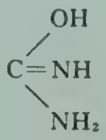
Aside from the effect of sodium hydroxide on viscosity and tack, it has the advantage of forming soluble sodium salts with compounds such as rosin, which are ordinarily insoluble in water. The incorporation of sodium hydroxide in an adhesive used for rosin-sized paper or board is therefore essential in order to form a satisfactory bond. A further advantage is the effect of alkalies on cellulose fiber in causing it to hydrate or swell, thus making possible better penetration of the adhesive. A disadvantage of these highly alkaline adhesives is the tendency to discolor the cellulose in paper, wood, or textiles, or to affect the color of the dye if the surfaces are dyed with a material that has chromophores that are sensitive to pH changes.

Next in importance to borax for the chemist working with adhesives is urea. Although it has been known for many years that urea has the property of "solubilizing" starch in the cold, the application of urea to adhesive bases is of recent origin (25). Urea, in its effect on starch bases for adhesives, has the opposite effect from that of borax. Borax increases the viscosity of dispersions of starch, dextrin, or British gum; urea decreases it. The property of urea to lower the viscosity of starch or dextrin dispersions has important commercial significance, for it permits the use at high concentration of starch derivatives that are not too highly fragmented or disorganized, thus producing adhesives of greater flexibility and bonding strength. Normally, adhesive bases that are not too highly fragmented would require too much water to form a satisfactory "solution." This results in low speed of operation, poor initial tack, etc. Through the use of urea, these bases can be used with smaller amounts of water, so that the speed of drying and the rate of development of tack are greatly increased. The effect of urea on starch products in dispersion is undoubtedly due to the interaction of chemical groupings. The mechanism, however, is uncertain. According to Werner (26), urea can exist in two forms; i.e., inactive and active.

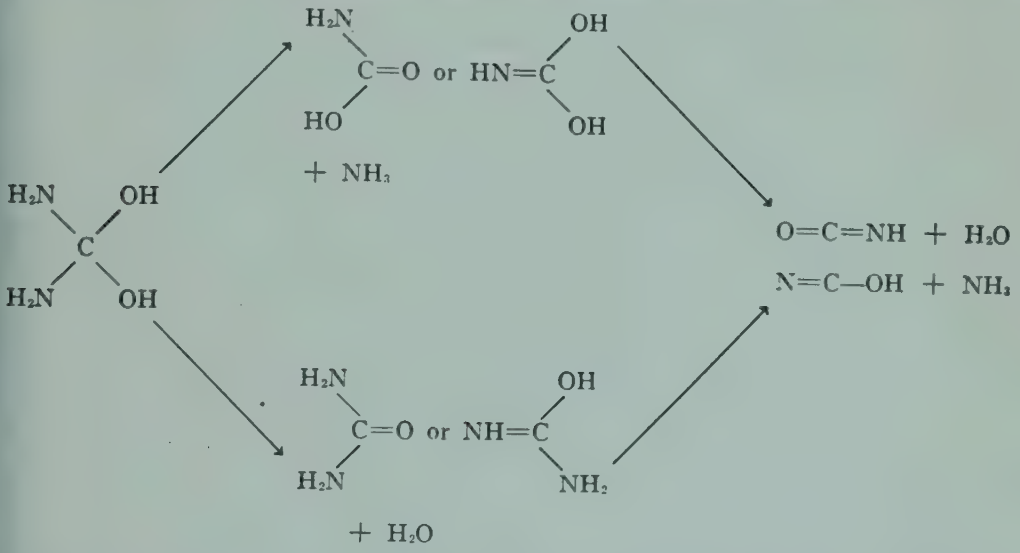
Inactive



Active



Blair (27) suggests the accompanying possible hydrated and non-hydrated forms of urea. It is possible that the primary bonds, particularly in the unsaturated states of urea, combine with the exposed groups of starch and starch derivatives more readily than these groups combine with water, thus displacing solvated water. At the same time, the hydrating groups of urea are fewer in number than those of the starch derivatives, thus leaving more solvent water.



Similar to urea in their general action, although each exhibits important differences, are other solubilizing chemicals, such as iodides, thiocyanates, chloral, etc.

d. Wetting Agents. The science of surface phenomena has made tremendous strides in the past 25 years. The interested student is advised to study the excellent treatises on the subject, and particularly the work of Langmuir and Harkins. Suffice it to say, here, that wetting or non-wetting of a liquid over the surface of another liquid or solid will depend on the surface and interfacial tensions of the substances in contact. If the surface tension of substance X (*i.e.*, the interfacial tension between X and the air or gas over its surface) is greater than the combination of the surface tension of substance Y plus the interfacial tension between X and Y, then X will wet the surface of Y; if less, X will not wet or spread over Y. Harkins has developed a method for the prediction of the ability of one liquid to spread over another, which is expressed by the formula, $S = W_a - W_c$ where S is the spreadability coefficient; W_a is the work of adhesion of the spreading surface over the surface to be spread; W_c is the work of the surface cohesion of the spreading material. The surface of one substance will spread over that of another if the work of surface cohesion is less than its work of adhesion with the surface over which spreading is attempted.

The use of wetting agents with adhesives helps the wetting of difficult surfaces and thus increases the specific bond. There are numerous wetting agents on the market. In selecting them, consideration must be given to the type of surface to be wetted and the composition of the adhesive. It should be borne in mind that the starch adhesive is aqueous and therefore the surface-modifying agent should have groups that can associate with water. Another point to be remembered is that too liberal an amount of these wetting agents affects the tack. The material most commonly used is sulfonated castor oil. Surface active materials such as Nacconol and similar compounds are also coming into use.

e. Plasticizers. A dry vegetable glue film is quite brittle. Even though starch micelles are considered to be made up of molecular chains arranged more or less in loop form, a condition which should tend to impart elasticity, the dry starch films are nevertheless far from flexible. As might be expected, dextrins and British gums which consist of fragmented starch micelles form films which are more brittle than those of the original starch. The starches and starch products made from the different starches differ somewhat in elasticity. Thus, potato, tapioca and waxy maize are considerably more elastic than corn or wheat, but at best the elasticity of the most elastic of the starches is comparatively poor. The brittleness of the vegetable glue film may be reduced somewhat by making the film thin. It is determined also to some extent by the surface on which the film is formed, but any further improvement necessitates the use of plasticizing agents.

The important requisites of plasticizing agents for starch adhesives are that the plasticizer be miscible with water, that it be compatible with the ingredients of the adhesive, and that it should not affect adversely the performance of the adhesive, at least not any more than can be tolerated safely by the particular adhesive to be plasticized. Most of the plasticizing agents used depend on their hygroscopic properties and a few on their fatty characteristics.

Chief among the plasticizing agents which depend on their hygroscopicity for flexibility are glycerol and the related products, the ethylene glycols. The dried film, containing a small percentage of glycerol or ethylene glycol, will hold tenaciously to the associated water molecules and thus will prevent the film from completely drying out and becoming brittle. Should the film become extremely dry, due to particularly arid atmospheric conditions and high temperatures, it will reabsorb moisture from the atmosphere when the humidity is increased. The moisture in the air and the moisture in the glycerol-containing film constantly tend to become equilibrated, and the conditions affecting this equilibrium are determined by the vapor pressures of the system, vapor-glue film, at any one time.

If the film retains or absorbs an excessive amount of moisture, the bond is weakened, and in extreme cases enough water might be present so that the bonded surfaces can be pulled apart without tearing the fiber. Should this excessive absorption take place on a surface film, such as envelope seals, labels, etc. "blocking" will take place. Excessive amounts of glycerol will also tend to reduce the rate of development of tack and the speed of adhesion. It is therefore extremely important to adjust the amount of glycerol added, so as to obtain flexibility without sacrificing strength of bond, rate of tack, or running the danger of "blocking." This quantity is not always easy to determine, since the amount of moisture in the film is dependent on atmospheric conditions.

Glucose and invert sugar are used as plasticizing agents. As with glycerol, the improved flexibility of the film is due to the moisture which it retains or absorbs. Invert sugar is considerably more hygroscopic than glucose and is therefore superior to it. Neither of them, however, is as good as glycerol or diethylene glycol, principally because they give up absorbed moisture too readily under dry atmospheric conditions and reabsorb it too slowly. Sorbitol is considered superior in these respects and its use has been advocated. The effect of the sugars on the bond, rate of tack, "blocking," etc. must be considered just as in the case of glycerol. The sugars have the additional disadvantage that they tend to develop color with age. This brownish coloration is undesirable in some applications.

The use of glycerol, ethylene glycols, sugars, etc., is not advisable with borated gums, since these products interact with the borax to form new products, the properties of which may not be consistent with the requirements of the adhesive. Other substances used as plasticizers because of their hygroscopicity are calcium chloride, ammonium nitrate, urea, sodium acetate, etc.

Sulfonated oils or fats, sulfated alcohols, and soluble soaps are examples of substances used for plasticizing in which the effect is not due to the retention or absorption of moisture. These substances tend to impart a permanent flexibility independent of atmospheric conditions. Their chief drawback is that generally the amounts necessary to give appreciable flexibility, say, about 3% of sulfonated oil, decrease the speed of tack and weaken the bond.

f. Defoamers. The foaming of adhesives is one of the most troublesome factors in adhesive operations. If foam collects in the gum box, the gum will not transfer properly onto the rolls; it will be full of air bubbles and poor in solids, the operating speed will be reduced, the bond will be poor, etc. It is therefore extremely important that adhesives subject to foaming contain defoaming agents to conteract the accumulation of foam.

Foams are dispersed systems of globular liquid films containing gases. These systems are formed in adhesives sometimes during the cooking, in which case the gas may be water vapor. More often, however, they are formed in use, owing to the incorporation of air into the surface of the liquid through the revolution of the rolls. If the systems are unstable, then the films break, and no accumulation of foam takes place. If they are stable, the foam accumulates and becomes troublesome. Development of foam and its relative stability are due to surface and interfacial forces and can therefore be greatly affected by the presence or introduction of surface-active materials.

The formation of foam is generally due to surface-active substances, foaming agents, which are present in the adhesive as impurities. Thus, the excessive foaming of adhesives made with a wheat starch base as compared with the foaming of adhesives made from tapioca is most likely due to traces of gliadin present in the wheat starch. The tendency to foam may be further increased by the viscosity of the dispersion, which causes air bubbles to be trapped and slows their rise to the surface where bursting and escape of the gas or air takes place. To prevent the formation of foam, other surface-active materials must be added which will modify the surface tension and interfaces in such a way as to counteract the action of the surface-active materials responsible for the foaming. Chief among the defoamers are alcohols, ethers, esters, fatty acids, oils, or fats. The Tweens and Spans of the Atlas Powder Company have been used successfully. The higher alcohols, such as capryl, heptyl, and nonyl are effective foam depressants. Their action is due to the fact that they form delicate films of low viscosity which tend to replace the more persistent films. They are effective in most types of solutions, particularly in those containing proteins, and exceedingly small amounts are sufficient to destroy accumulated foam and prevent its re-formation.

Oleic, stearic, and palmitic acids are effective foam depressants in many cases. Vegetable oils and sulfonated vegetable oils are also useful. The effects of these substances may be due either to differences in surface tension between the surface-active materials (*e.g.*, the added substance may lower the surface tension more than the substance already present at the interface and will displace it from the interface) or to the presence of polar and nonpolar groups which take a definite surface orientation, or to both. The amounts of these materials necessary to prevent foaming may vary from 0.1 to 0.5%, based on the weight of the solids. The amount is rather important, as in some cases an excessive amount of defoamer will cause re-formation of foam. The phase may change from one consisting of water saturated with the defoaming agent to that of the defoaming agent saturated with water.

Tributyl phosphate and tributyl citrate have also been used successfully to prevent foaming, in the amounts of 0.1 to 0.25%.

g. Preservatives. Preservatives are generally unnecessary in aqueous adhesives when the percentage of solids is high. Envelope adhesives, containing about 65% of solids, seldom, if ever, require a preservative. Apparently there is not sufficient free water available to encourage the propagation of the stray molds or bacteria that may enter it from the air or from the vessels or materials used.

Adhesives that contain appreciable quantities of chemicals, such as borax, even though they may be made up with a fairly large amount of water (70 or 80%) are also seldom subject to the action of molds and bacteria. Nevertheless, there are occasions when even envelope gums or borated gums become favorable substrates for the activity of bacteria and molds. This is evidenced by a lowering of the viscosity and the development of a sour odor. The infection is due to the presence of a particularly virulent strain of bacteria or mold with which the adhesive dispersion has become contaminated, or one which has been cultured in the residues of the adhesive in the containers and has developed high tolerance to that particular composition. The usual remedy in such cases is to clean thoroughly all the vessels used in preparing and storing the adhesive with a hypochlorite or other such bactericidal compound.

When the concentration of solids is low (*e.g.*, less than 30%) and no chemicals are present which create an unfavorable medium for living organisms, preservatives should be incorporated, particularly if the adhesive is not used up within a short time. It is extremely difficult to make definite recommendations on the type of preservative substances to be used. This is readily understandable when we consider the great variety of strains of molds and bacteria which vary in their response to different chemicals. What may be toxic for one type of organism may be entirely harmless for another. It is suggested, generally speaking, that a preservative be used which will affect both molds and bacteria, and if only one substance will serve each purpose for a particular solution, then two different chemical agents should be used, one specific for molds and the other for bacteria.

Of the various chemical compounds, mercurials are among the best. They are effective against most types of organisms. Chlorinated phenols are quite effective. A small amount, 0.2% of the sodium salt of *o*-phenylphenol (Dowicide A) or of the sodium salt of pentachlorophenol (Dowicide G) offers good protection. Formaldehyde, in percentages of 0.5 to 1.0, is widely used. It is particularly effective against molds. Copper sulfate, to the extent of about 0.2%, zinc sulfate, fluorides, benzoates, and phenols are frequently used (28).

In selecting the proper preservative, consideration should be given not only to the protection of the adhesive, but also to the possible toxic effect of the particular chemical on the operator in the plant and the possible implications due to its presence in the finished product.

h. Color. The color of adhesives is of some importance when they are used on exposed surfaces, such as on labels, envelope seals, etc. When the adhesive is used for bonding two surfaces, color is of little concern unless the laminated material is white and it is important not to reduce the whiteness through the use of a dark adhesive.

The color of the adhesive can be regulated to a large extent during the manufacture of the base. However, if adjustments in color of the finished adhesive are necessary, darker shades may be produced by the use of caramel coloring, and lighter shades by bleaching. The more effective bleaching agents used are sodium bisulfite if the medium is acid, or perborate, if the medium is alkaline. From 0.1 to 0.25% is generally sufficient. Hydrogen peroxide is also widely used.

i. Emulsions. Mention has been made before that the incorporation of sodium hydroxide into an adhesive solution is of considerable help when the surfaces to be adhered are sized with rosin. In such cases, the alkali acts on the rosin by forming a soluble salt which disperses through the solution, thus permitting the adhesive to reach the paper fibers. When the paper or board is sized with oil, wax, insoluble soaps, or synthetic resins, alkalies may have little effect. In such cases it is necessary to incorporate a solvent which will dissolve the oil, wax, or soap layer and thus expose the paper surface. The solvent to be selected should be one that will solubilize the particular material with which the paper or board is sized, and preferably one that is not miscible with water. A solvent that is miscible with water is apt to be too highly diluted with the water to be effective, unless large amounts of it are used. Best results are obtained through the use of an immiscible solvent by emulsifying it with the aqueous adhesive into an emulsion that does not possess high stability. When the adhesive is applied to the surface, the change in the system due to evaporation, etc., is sufficient to break the emulsion and permit the solvent to act independently on the surface to dissolve off the protective film. As an illustration, it is possible to form a good bond with an aqueous adhesive on a waxed surface by incorporating a small amount of carbon tetrachloride (about 10%) and emulsifying it with sulfonated castor oil (about 1%).

j. "Fluidifying" Agents. Many adhesive solutions tend to "paste back" and lose fluidity within a short time after they are prepared. This is particularly troubleome when the base of the adhesive is a white dextrin or a thin boiling starch. A pasty adhesive cannot be used in operations for which a low viscosity is necessary, and if this "pasting back" occurs before the adhesive is used up, it is a source of many difficulties for the operator. Frequently, a solution may thicken gradually for several days before completely "pasting back." In such cases the machine operator will attempt to dilute the viscous solution with water in order to bring it to the right viscosity for machine operation. This, however, lowers the content of solids of the diluted adhesive, reduces the speed of tack, and slows up operation.

To overcome this tendency to "paste back," and to maintain greater uniformity in the viscosity of the adhesive solution during its life, various chemical substances can be used. Salicylic acid and related compounds are quite effective, even in small percentages. However, because of their phenolic nature they usually develop color, which is objectionable in many cases. They are also comparatively costly.

Formaldehyde in the proportions of about 2 to 3% is also effective in some cases. Boric acid, up to 5%, is widely used. It has been claimed that inorganic salts, such as potassium chloride, sodium sulfate, etc., serve to keep a solution fluid. Acetamide, dicyandiamide, and related amides have been advocated, while urea, potassium iodide, and thiocyanates also have been found of definite value in minimizing changes in viscosity in adhesive solutions.

The underlying principle is most likely due to the reactive groups reacting in such a manner that little association with water can take place, thus leaving the free water to act as solvent.

4. Conclusion and Acknowledgment. It has been quite a task to

condense the subject of starch adhesives into the space of one chapter. It has necessitated discussing briefly subjects that merit more detailed treatment. In particular it is regretted that more space could not be devoted to the relationship between the adhesive and machine and the importance of coordinating the one with the other. It is hoped, however, that this chapter will serve as a stimulus to the chemists working on starch adhesives for a more scientific approach to problems concerning adhesives.

Appreciation is expressed to Mr. J. V. Bauer for his communication on corrugating adhesives, and to Mr. John Mahlmann for gathering some of the references cited.

BIBLIOGRAPHY

1. Brown, F. R., and Truax, T. R., in Weiser, H. B., Colloid Symposium Monograph, The Chemical Catalog Company, New York, Vol. 4, p. 258 (1926).
2. Graham, T., *Proc. Roy. Soc. London,* **13**, 335 (1864).
3. Thomas, A. W., Colloid Chemistry, McGraw-Hill, New York (1934).
4. Bancroft, W. D., Applied Colloid Chemistry, McGraw-Hill, New York, p. 74 (1921).
5. McBain, J. W., and Hopkins, D. G., *J. Phys. Chem.,* **29**, 188 (1925)
6. Bikerman, J. J., *Chem. Age,* **47**, 186 (1942).
7. Third Report of the Adhesive Research Committee, G. B. Department of Science and Industrial Research (1932).
8. McBain, J. W., and Lee, W. B., *Proc. Roy. Soc. London,* **A113**, 606-620 (1927).
9. Clark, G. L., in Weiser, H. B., Colloid Symposium Monograph, The Chemical Catalog Company, New York, Vol. 4, p. 169 (1926).
10. de Bruyne, N. A., *Flight and Aircraft Engineer,* **36**, suppl. 12, 534a (1939).
11. von Buzāgh, A., *J. Phys. Chem.,* **43**, 1003 (1939).
12. Schnurmann, R., *J. Appl. Phys.* **13**, 235-245 (1942).
13. Bikerman, J. J., *J. Soc. Chem. Ind.,* **60**, 23-24 (1941).
14. Stamm, A. J., and Seborg, R. M., *Ind. Eng. Chem.,* **28**, 1164 (1936).
15. Houtz, C. C., and McLean, D. A., *J. Phys. Chem.,* **43**, 309 (1939).
16. Dulac, R., and Macfarlane, W. S., translated by Rosenbaum, J. L., Industrial Cold Adhesives, C. Griffin and Company, London (1937).
17. Bauer, J. V., U. S. Patent 2,051,025; 2,102,937; 2,212,557.
18. Bauer, H. F., U. S. Patent 2,215,847. Edson, E. R., and Mach, G. F., U. S. Patent 2,210,119.
19. Bauer, H. F., Bauer, J. V., and Hawley, D. M., U. S. Patent 2,144,610; 2,145,195.
20. Bauer, H. F., Bauer, J. V., and Hawley, D. M., U. S. Patent 2,167,629.
21. Bauer, J. V., and Hawley, D. M., U. S. Patent 2,197,754.
22. Army-Navy General Specifications for Packaging, Packing, and Marketing for Overseas Shipment; Oct. 10, 1942, U. S. Army No. 100-14, Chapter VIII, Solid Fibreboard Shipping Boxes, p. 54a.
23. Caesar, G. V., U. S. Patent 2,283,044.
24. Zulkowski, K., *Forstchr. von Chem. Ind.,* **22**, 280 (1900).
25. Bauer, H. F., U. S. Patent 2,215,846; 2,215,847.
26. Werner, E. A., Chemistry of Urea, Longmans, Green, London (1923)
27. Blair, J. S., *J. Am. Chem. Soc.,* **48**, 87 (1926).
28. Frieden, A., *Paper Trade J.,* **110**, 29-32 (1940).

CHAPTER XXIII

MISCELLANEOUS USES FOR STARCH

The varied uses for which starch, or some modification of starch, is employed are broadly outlined in Table XXXVI. In this table the type or types of products used in the application is also indicated, although generalizations of this nature should not be taken to signify that types other than the ones shown cannot be used under the proper conditions. Some of these industries may employ processes sufficiently different among themselves or may use such varied sources of raw materials or intermediates that a starch product suitable for one mill may be quite unsatisfactory in another.

Several of the more important uses for starch have been discussed in previous chapters. Others, which cannot be classified under these headings, will be discussed in the following pages.

1. **Ceramics.** Recently, the effect of starch in modifying the colloidal properties of clays has been extended to the field of ceramics. When china clay is mixed with water, a mass results which has relatively high plasticity and other characteristics adversely affecting the workability. Normal practice is to add to the china clay between 5 to 10% of a special plasticizer called "ball clay." However, "ball clay" may contain impurities which adversely affect the characteristics of the fired ceramic. For example, the presence of iron and titanium compounds cause the bodies to fire to an off-shade of color. Furthermore, adjuncts of this nature contribute little to the dry strength of the china clay body before firing.

The practice in the past has been to add minor amounts of adhesives such as starch and dextrins to act as a binder. Recently, (1) the effect of larger additions of starch products (1-3%, based on weight of clay) has been studied with a view to securing a plasticizing effect and to replace the "ball clay." It was found that whereas 8% ball clay reduced the plasticity, as measured by flow point load, 50%, 3% of starch lowered the plasticity 80%. Moreover, the starch increased the dry bond strength 4.5 times and gave a much whiter finished ceramic.

Naturally, unmodified starches hold a large ratio of water on gelatinization and their use increases the moisture content of the clay body, which, in turn increases shrinkage during drying and porosity during firing. The use of an oxidized starch or dextrin (British gum type) is indicated.

Ellis-Anwyl (2) has reported that in the processing of clays at 180°F. for brick and tile manufacture, the addition of gelatinized starch produced comparable results. Workability was improved as well as the binding and drying qualities of some of the clays studied.

TABLE XXXVI

Uses for Starch and Starch Products

Industrial use	Unmodified Starch or Derivative	Enzyme Converted Starch	Dextrin	Pregelatinized	Thin Boiling	Oxidized
ADHESIVES						
Bill Posting	x		x	x		
Corrugated Paper	x				x	x
Envelopes			x			
Laminated Solid Fibre		x	x		x	x
Paper Bags	x	x	x		x	x
Paper Boxes (lining)			x	x	x	
Paper Tubes & Cones	x		x	x	x	
Postage Stamps			x			
Gummed Tape			x			
BATTERIES, DRY CELL	x					
BOILER COMPOUNDS	x					
BRIQUETTS						
Coal	x			x		
Crayons (chalk)			x		x	x
Metals	x	x	x			
CERAMICS	x	x	x	x	x	x
CHEMICALS						
Organic Acids	x					
Organic Solvents	x					
CIGARETTE SEALING	x	x				
CORD POLISHING	x	x	x			
CORK PRODUCTS	x		x		x	
COSMETICS						
Hair Waves	x					
Shaving Cream	x					
Powders	x					
Tooth Paste	x					
DETERGENTS	x			x		
DYES			x			
EXPLOSIVES						
Dynamite	x					
Fireworks	x					
Nitrated Starch	x					
Various	x			x		
FOODS						
BAKERY						
Cakes	x					
Cookies	x					
Dusting	x			x		
Flours	x					
Ice Cream Cones	x					
Macaroni	x					
Pie Filling	x					x

TABLE XXXVI (*continued*)

Industrial use	Unmodified Starch or Derivative	Enzyme Converted Starch	Dextrin	Pregelatinized	Thin Boiling	Oxidized
Prepared Mixes	x			x		
Wafers	x					
BREWING	x	x		x		
CANNED GOODS						
Canned Vegetables	x					
Soup	x					x
Thickeners	x					
CONFECTIONERY						
Caramels	x					
Chewing Gum	x			x		
Drageé	x		x			
Dusting	x			x		
Gum Work	x				x	x
Molding	x					
Pan Coatings			x	x		
DAIRY FOODS						
Condensed Milk			x			
Stabilizers	x		x			
DESSERTS						
Pudding Powders	x			x		x
MEAT PRODUCTS						
Sausage and Loaf Binders	x			x		
MISCELLANEOUS						
Animal Foods	x		x	x		
Baking Powders	x					
Condiments	x			x		
Rice Polishes			x			
Salad Dressings	x					x
Sugar, Powdered	x					x
Yeast	x					
Films	x					
OUNDRIES						
Sand Binder			x	x		
NSECTICIDES	x		x			
NSULATIONS						
Glass Wool	x					
Rock Wool	x		x			
AUNDRY						
Bluing	x		x			
Commercial	x				x	x
Home	x	x		x	x	x
EATHER						
Adhesives			x			
Shoe Counter Pastes			x		x	
Shoe Polishes	x		x			

TABLE XXXVI (*continued*)

Industrial use	Unmodified Starch or Derivative	Enzyme Converted Starch	Dextrin	Pregelatinized	Thin Boiling	Oxidized
LINOLEUM			x			
LUMBER TREATMENT	x					
MINING						
Ore Flotation	x				x	x
Oil Well Drilling				x		
Process Control	x		x	x		
Settling	x			x		
PAINTS						
Cleaning Compounds	x			x		
Cold Water Pastes			x	x		
Poster	x		x			
Sizing	x					
PAPER	x			x		
Beater Sizing						
Calender Sizing	x	x	x		x	x
Coatings	x	x	x		x	x
Tub Sizing	x	x	x		x	x
Wall Paper		x	x		x	x
PHARMACEUTICALS						
Starch Tablets	x					x
Surgical Dressings				x		
PLASTICS						
Miscellaneous	x		x	x		x
Extender	x					
Molded	x		x			
Coatings	x		x			
PLYWOOD AND VENEERS	x					x
PRINTING	x		x			
PROTECTIVE COLLOIDS						
Emulsions	x		x	x	x	x
RAYON MANUFACTURE	x		x			
ROAD PAVING	x					
RUBBER						
Battery Plates	x					
Dusting	x					
SILVERING COMPOUNDS	x		x			
SOAP MANUFACTURE AND DETERGENTS	x		x	x		
TEXTILES						
Cloth Printing	x		x			x
Finishing	x	x	x	x	x	x
Rug Sizing		x	x			
Screen Printing			x			
Stiffening	x		x		x	
Warp Sizing	x	x			x	x
WATER TREATMENT				x		
WINDOW SHADES	x		x			

Hoggatt (3) has patented the use of starch to improve the physical and mechanical properties of plaster casts.

2. Cosmetics, Pharmaceuticals and Insecticides. Starch has long been used in the preparation of cosmetics, pharmaceuticals, germicides, insecticides and related uses. Indeed, one of the oldest uses for starch is that of a dusting powder. It is reported that the principal use for starch during the 17th and 18th centuries was for the powdering of wigs. Starch has also been used as a face powder. With the development of fine talcs and other mineral powders, the use of starch as a powder for applications on humans has declined.

Applied externally, talc powders have some advantages over starch. Some of the newer powders have greater covering power, for example, and the surface does not present a dusted or powdered appearance which is no longer desirable. However, in other cases, as for example, in surgical operations, the introduction of mineral powders into wounds may set up an irritation and possibly induce the formation of lesions owing to the difficulty the system has of disposing or assimilating these powders. For example, surgeons use relatively large amounts of dusting powders on the inside and outside of rubber gloves worn during operations. There are several reasons for this, among which are the lubrication of the gloves while in the sterilizer, and lubrication of the hands so as to permit nimbleness and flexibility of the fingers and hands to the greatest degree. Accordingly, appreciable amounts of these powders may often be introduced into wounds. An assimilable powder would be a boon in this case and starch has been used for this application. Native starch has one serious disadvantage. The powder obviously must be unaffected physically by steam sterilization, and starch tends to gelatinize under these conditions and become sticky. The author has experimented for several years and has produced nongelatinizable starches by several means but the problem has not been solved with complete satisfaction. As the starch becomes completely nongelatinizing, *e.g.*, by reaction with bifunctional agents such as epichlorohydrin or by treating with formaldehyde either with acid in the wet state or in a gas-solid phase reacting system, the starch becomes nonassimilable, as might be anticipated. If the reaction is stopped just short of the point where it cannot be assimilated, then the starch gelatinizes just sufficiently so as to form a gritty texture on the rubber goods during sterilization. Accordingly, a compromise is usually adopted and the reaction is extended to a level just intermediate to the two points indicated above. Apparently this compromise is acceptable and preferred by some of the medical profession to other dusting powders (4).

The powder is most readily made by reacting starch with formaldehyde, or formaldehyde liberating reagents, by an adaptation of the processes outlined many years ago by Classen (5, 6), Herstein (7) and others, and recently defined by Pierson (8). Classen claimed the use of insoluble starch prepared by the formaldehyde reaction as a surgical dressing.

An absorbable surgical dressing base would be a distinct advantage since if left in a wound or incision, reopening of the wound to remove the dressing would not be necessary, and if used as an exterior wet dressing, then tissue forming about pieces of the dressing would not need to be cut. Such a dressing has been described by MacMasters and Blom (9) who utilized the observation of earlier workers, such as Ling and Nanji (10), that when starch pastes are frozen and thawed, a large portion of the starch remains insoluble as a waddy mass, which is sponge-like in character after pressing or washing. MacMasters and Blom suggest that drugs such as sulfathiazole may be added to the starch paste before freezing. Also, as pointed out by Bice *et al.* (11), since these sponges absorb between 15 to 18 times their weight of water or alcohol and are firm enough to stand handling, medicinals such as penicillin can be introduced into the sponges by dipping into a solution of the drug and drying. During enzymic breakdown of the sponge in the body, the medicament is released. This application has been patented by Bice and MacMasters (12).

One of the older applications for starch in pharmaceuticals has been the use of starch powder as a diluent, binder or carrier in the preparation of compressed tablets for internal use. Many drugs are too powerful in physiological action to be made into tablet form having a size large enough to be conveniently handled. Starch is frequently added to give the desired bulk. The medicament may be mixed directly with the starch or first diluted with a suitable solvent and the solution mixed with starch or other carrier. Some drugs compress too firmly, or are too tacky, so as to disintegrate in water or body fluids at a desirable rate. The addition of starch in small amounts as a disintegrating agent has frequently been employed. Spengler and Jud (13) have discussed some of the problems involved and have given a series of practical directions. Corn, arrowroot, or potato starch is employed, depending on the type of material to be made into a tablet.

More recently, starch derivatives have been used as the active agents. A patent has been issued to Karrer (14) describing an anticoagulant for blood. Starch, or cellulose, is partially etherified with a β-haloethanesulfonic acid and partly esterified with a halosulfonic acid, and the acid ester groups are converted to an alkali salt. Almost simultaneously, Walter (15) was granted a patent for an inhibitor for blood coagulation made by reacting part of the hydroxyl groups of a polysaccharide with a halosulfonic acid and etherifying with a halocarboxylic acid, *e.g.*, choroacetic acid, in alkaline solution.

Husemann *et al.* (16) studied further the action of blood anticoagulants prepared by the method of Karrer. It was found that the sulfuric acid esters of highly branched starches or glycogen had little or no effect. Further indication that linear polymer derivatives are more effective than branched was shown from experiments wherein polyvinyl alcohol and cellulose derivatives showed a marked effect also. Both the anticoagulating effect and the lethal dose increased with decreasing molecular weight over the range 300,000 to 20,000. Presumably, therefore, the ester of an amylose such as that of the corn crystalline amylose prepared by Kerr and Severson (17) would be representative of a starch product having the optimal properties for this purpose.

Minto (18) has claimed that the complex formed by amylose with iodine has therapeutic properties.

Starch has been used to improve the colloidal properties and the stability of pharmaceutical preparations. Termansen (19) has given experimental data on the use of starch for this purpose and has discussed methods for preparing starch products from several sources.

Starch and water soluble starch derivatives are used in hand lotions and in protective skin lotions such as might be used to safeguard against occupational dermatitis. Khesin (20) has described the preparation of a lotion of wheat starch and gelatin with Burov's reagent in glycerol and suggests that it is effective in protecting the skin against exposure to petroleum and coal tar or their distillates, turpentine, lacquers and organic dusts.

Borglin (21) has patented starch thiocyanates, thiocyanobutyrates, selenocyanoacetates, tellurocyanoacetates, and similar compounds as being useful as insecticides, particularly for killing larvae of the house fly and moths. Brown (22) has patented the use of partial capric or lauric acid esters of carbohydrates as insecticides. A patent has been allowed to Gaver (23) for mildewproofing agents made by reacting sodium starch with salts of heavy metals such as copper.

3. **Detergents.** Starch products are useful in two ways in detergents. In one, an ungelatinized, water-insoluble starch is used and the starch granules function by virtue of their adsorption capacity for fats, grease and soil. In the other, a water-soluble starch product is used in order to assist the primary detergent in keeping dirt in colloidal suspension so that it does not redeposit in the articles which are being cleaned.

The first mentioned use is the older of the two. For many years powdered starch has been added to soap in the formation of the cakes, particularly in European manufacture. As high as 10 to 15% has been added, although this application has declined rather than expanded in recent years. However, compounded detergent powders, containing raw starch, are manufactured in some volume. These are used for cleaning painted surfaces such as woodwork and walls and in the dry cleaning of furs, lacquered finishes such as those of automobiles, and related uses. For the wet cleaning of painted surfaces a mixture is made primarily of a soap powder (80%), a buffer such as sodium dihydrogen phosphate (1%) and powdered starch (19%). In "dry" cleaning, the starch may be worked into the fur with a solvent such as chlorohydrocarbon, the excess solvent removed and the starch then shaken from the fur when it is dry.

The second mentioned use has expanded with growth in the use of synthetic detergents. Fatty acid soaps are not only fair detergents, but they also tend to keep soil suspended colloidally. Their chief disadvantage is that their efficiency is greatest in soft, neutral or slightly alkaline water. Calcium, magnesium and other metals found in some waters tend to form insoluble fatty acid salts, thus reducing the efficiency of the common soaps. Many of the newer detergents operate effectively in hard water. With some, their detergent action may surpass the fatty acid soaps but, on the other hand, their suspending power for soil may be considerably less. It is with this latter class that the addition of a suitable starch product is most beneficial.

To obtain this colloidal effect with starch, it must be gelatinized or made cold water dispersible. Products used initially were of the roll-dried type of starch with little or no modification. More recently, improved products have been available and these are the cold water soluble derivatives or their salts, such as carboxymethyl starch.

Höppler (24) has pointed out the use of sodium starch glycolate or carboxymethyl starch with detergents. Nieuwenhuis (25) found that a small addition of carboxymethyl starch increased to some extent the suspending power and over-all washing efficiency of a fatty acid soap, but the same amount of carboxymethyl starch very greatly improved the suspending action and over-all washing efficiency when the Na salt of a monosulfate of aliphatic alcohols (10-18C atoms) was used as the detergent.

Nusslein and Pauser (26) have claimed the use of water-soluble derivatives of starch with ether linkages to hydrocarbon radicals substituted by hydrophilic groups when mixed with a minor amount of surface active agents, which would not of itself be sufficient to effect washing.

4. Emulsifying and Dispersing Agents. A large outlet for starch as an emulsifying agent is in the manufacture of salad dressings. This use has been discussed under the heading of foods. Petersen (27) has recently

claimed a superior starch dispersing agent for oils and fats by esterifying the starch with fatty acids containing more than eight carbon atoms. Gaver (28) has patented an improved emulsifying and dispersing agent formed by treating starch in alcoholic suspension with caustic alkalis. The alkali metal starchate is dispersible in water. In a second patent, Gaver (29) describes the preparation of wax emulsions in water. The colloidally dispersed wax particles are coated with sodium starchate. In another instance, Gaver (30) discloses the use of sodium starchate in the preparation of a paint which is composed of a varnish emulsified with an aqueous solution of sodium starchate in which the pigment is dispersed.

Groen and Krizkovsky (31) have patented a composition consisting of roll-dried potato starch, ground and powdered and mixed with an equal weight of skim milk solids. It is claimed that the mixture may be beaten in water to produce a mass resembling whipped cream.

5. **Explosives.** Starch has been used for many years in the manufacture of explosives, either as the primary active ingredient or as an adjunct. Starch nitrate has been used primarily as a demolition agent. Its principal fault has been its great instability which has materially restricted its use and which property has been the principal subject of research in this field for several years.

Ashford *et al.* (32) investigated the relative stability of the nitrates prepared from amylose (adsorbed by cotton) and amylopectin and concluded that the linear polymer nitrate approached the stability of cellulose nitrate, whereas the branched polymer nitrate was less stable. The opinion has prevailed that various impurities associated with the starches such as fatty acids, or sulfates, initiate this instability (33). Several patents have been granted (34-37) based on the conclusion that a rigorous purification of the starch and the nitrate leads to greater stability. More recently, however, the importance of trace amounts of inorganic acids in the end product has been emphasized. Berl and Kunz (34) have nitrated dry starch in 100% phosphoric acid with nitric acid at 0°C. and claim that a relatively stable product results. Instability was thought to be due to traces of H_2SO_4 which cannot be removed from the product when nitration is accomplished with H_2SO_4-HNO_3 mixtures. Caesar and Goldfrank (38) have favored the elimination of all acids including nitric. These workers have nitrated dry starch with nitrogen pentoxide in CCl_4 in the presence of an agent which binds HNO_3, through hydrogen bonding, as soon as it is formed. A fluoride, such as NaF, is recommended for this purpose. After washing the fully nitrated product with water and dilute NH_4OH, a relatively stable product results.

Snelling (39) has patented a composition composed of nitrostarch,

$Ba(NO_3)_2$, coal dust, Al powder and other adjuncts, as a demolition explosive. Snelling (40) has also patented a composition which is sufficiently stable to be insensitive to a No. 6 blasting cap. Aggregates are coated with starch nitrate in the form of a powder and the latter may be bound to the aggregates by use of lacquer, resin or a corn sirup.

The use of various starch products in the manufacture of explosives is illustrated in several recently issued patents. Carey (41) has patented the use of starch, such as corn starch, with water-soluble nitrates, such as NH_4NO_3. The proportion of starch used is insufficient to combine with all the oxygen liberated from the nitrate but sufficient to bind the NH_4NO_3 particles together when a plastic mass of the two is dried sufficiently to form case-hardened agglomerates. Du Pont *et al.* (42) have patented a detonating explosive in which a flaked corn product, capable of forming a cohesive paste on contact with cold water, is mixed with nitrates and liquid nitric esters. It is claimed that the effectiveness of the explosive on immersion in water is improved.

Gaver (43) has prepared a low density explosive in which nitroglycerin is adsorbed on an alkali metal starchate.

6. Laundry Starches. One of the oldest and undoubtedly one of the most important uses for starch has been and is for the sizing of textiles. This general subject has been discussed broadly in another chapter. Moreover, starch manufacturers, for many years, have made specially modified starch products for sale not only to large commercial laundries but for home laundry use as well. These have been used for refinishing textile fabrics after a cleaning operation which ordinarily removes a large part of the original size in the textile. These products consist of a wide variety of starches such as corn, wheat, and rice, either singly or mixed, and in various states of modification resulting usually either from acid treatment or oxidation, depending on the specific sizing use intended or the preference of the manufacturer. These modifications, if any, are usually applied to the starch at a temperature under the gelatinization point and the product is finished as a powder, lump or tablet of ungelatinized starch. These have been sold under nationally and internationally known trade marks and need no elaborate description. In using products of this type, the laundry operator prepares either a cold water slurry, as for cold water starching, or cooks the starch with water in order to form a better dispersed starch size. Recently, starch manufacturers have developed several new types of laundry starches, two of which are sufficiently different from the older types to merit a discussion of their properties.

The principal purposes for using laundry sizes are to improve the body and appearance of the fabric, to impart a film on the fibers in order to protect them from wear and to impede the penetration of soil and dirt into the fabric, thus facilitating the next cleaning operation. When a high degree of body or stiffness is desired, an unmodified, or native starch is usually employed. Such is the case, for example, in the starching of collars, cuffs and similar articles. Usually the finish is board-like and has a high gloss unless softening agents, such as waxes, sulfonated oils or other adjuncts, are incorporated with the native starch. When less body is desired, the starch is pretreated with acids or oxidizing agents. Then when these treated starches are gelatinized, they break down on cooking into smaller colloidal units which tend to penetrate into the fabric rather than remaining as a thick film on the surface. A moderate amount of penetration and a limited amount of stiffness is desirable in the starching of linens, for example.

Naturally, starch in the ungelatinized state has no sizing value and develops this property as it is gelatinized and dispersed in a fine colloidal state. This requires cooking and use of the pasted starch before retrogradation effects such as skinning, reassociation of colloidal units into large masses and insolubilization, have become pronounced. However, cold water starching appears to be a desirable procedure, primarily due to convenience, no doubt, and has been used by many. In using starch products available in the past, the garments are immersed in a suspension of ungelatinized granules, the excess is squeezed out and the fabric containing a relatively large amount of water is finished with hot irons. The heat of the irons has been relied upon to gelatinize the starch in the fabric. Penetration of the whole starch granules into the fabric is usually poor and may be quite uneven. Moreover, gelatinization of the starch must, of necessity, be limited and this method does not provide for the most efficient use of starch as a size. In order to improve penetration of the starch into the fabric, starches consisting of relatively small granules have been used. Rice starch, for example, is generally thought to be superior as a cold water starching powder because of its very small granule size. Also, granules of small size settle from suspension in water less readily than large granules. Therefore, the use of suspensions of smaller granules requires less frequent stirring in order to maintain a more uniform distribution and this may promote a more even application of the starch. In order to cause a greater degree of gelatinization with the limited amount of heat provided in ironing, some of these starching powders are pretreated so as to lower their gelatinization temperatures, or alkaline substances such as borax may be mixed with the starch.

A novel type of cold water starching powder which contains only 40% of ungelatinized starch is described in two German patents issued to Reschke

(44, 45). A mixture is made of approximately 40% of potato starch and 60% of a cold water-swelling starch, borax and urea. The urea is claimed to affect the swelling of the starch. The cold water-swelling starch no doubt tends to hold in suspension the large, raw potato starch granules, when the product is mixed with water for use. Borax in the starch may be replaced by alkali sulfates.

Recently, manufacturers have developed two new laundry starches which may be used simply by mixing with cold water but nevertheless give the quality of and sizing efficiency obtained by cooked starch size. Both products are pregelatinized. The first type, of which at least a half-dozen trade brands have appeared on the market, is a liquid product (46). These cooked pastes have been found to contain between 5 to 15% dry solids, depending on the brand, and the starch has been modified to a suitable viscosity level for laundry sizing work. Set-back, freedom from gel formation and general colloidal stability, have been attained by the addition of adjuncts. Preservatives are usually added also.

One of the first liquid laundry starches to make its appearance was patented in 1941 (47). The process for making this nonseparating liquid starch consists in boiling approximately 12 oz. of starch in 1 gallon of water for not more than 3 minutes, cooling to not lower than 160°F., adding thereto and stirring in for about 3 minutes approximately 0.5 oz. of NaF (in 4 oz. H_2O) and finally adding 15 g. of pine oil.

A more recently issued patent (48) outlines a method for preparing a liquid laundry starch as follows: Corn starch is cooked until substantially all the granules are ruptured. The paste is cooled and the gelled mass is homogenized in the presence of pine oil and boric acid. Bluing is added also.

Peters (49) discloses a procedure to make a semi-liquid starch dispersion by cooking about 100 to 120 g. of starch in about 700 ml. of water, adding about 5 g. of stearic acid, 6 ml. of triethanolamine, 10 ml. of beta-pine oil and a preservative such as sodium benzoate and diluting to 1 liter.

Although these liquid products provide the consumer with the convenience of cold water mixing and obviate the necessity for cooking, nevertheless they possess the obvious disadvantage that the manufacturer must merchandise and the consumer buy a relatively large amount of water with the starch. This is because it is not possible, at present at least, to prepare a permanently fluid product from starch which has been degraded to the level where a very high solids concentration can be employed and where a good textile sizing starch will result. If the starch is hydrolyzed, oxidized, or dextrinized to the extent where the pasted product is fluid at high concentration, e.g., 40 to 50% in water, then one must use more liquid product and more dry substance starch to obtain an equivalent sizing effect than when the starch is converted to a lower fluidity level, common to starch for sizing purposes. The fluidity range of sizing starches extends from unmodified to about 40 to 60.

The second type of product is a dry powder which apparently has been premodified to the proper level, gelatinized in water, and dehydrated. Many dehydrated, gelatinized starches have been marketed in the past. Disadvantages attending their use as a laundry starch have been that they were either not freely dispersible in cold water or if they were sufficiently dispersible and cold water-soluble because of an extensive premodification, then they were weak in sizing value. In the past, therefore, use of products of this type has been limited to various applications wherein ample agitation is provided for the thicker varieties such as, for example, sizing paper pulp at the beaters. The thinner varieties which possess a relatively high rate of dispersibility in cold water and high content of cold water-soluble material because of an extensive premodification treatment have found application mainly in the field of adhesives. The incorporation of the two properties, high cold water-solubility with almost instant dispersibility and high textile sizing value in a starch product, is consequently a noteworthy development in the science and technology of starch. The ready acceptance of these new laundry starches by the trade indicates a preference, at least by the domestic user, for a prepared product which requires no cooking. It may indicate that these new products will displace a considerable proportion of the older types of laundry starches.

7. **Mining and Metallurgy.** During the past decade, mining and metallurgy have expanded their use of starch products at a greater rate than any other group of industries. Although a part of this very large expansion is attributable to the wartime expansion of foundries and the established use of starch products in this phase of the fabrication of iron, nevertheless, the novel uses for starch which were developed during this period not only augmented the total amount of starch used by these industries to a degree which seriously overtaxed starch manufacturers but these new uses promise to overshadow many other uses in the years to come.

The more important of these novel applications appears to be the drilling of oil wells, the refining of aluminum from bauxite and the floation of ores.

a. Drilling of Oil Wells. Starch products may be used in several operations concerned with the drilling of oil wells. Of these, the more important are the addition of a starch product to the drilling mud used in drilling the hole, to the drilling fluid used in cutting cores and to the cement which may be used to case certain sections of the well.

In the drilling of oil wells, a fluid called drilling mud is circulated downward through the hollow drill pipe, across the face of the drill bit and upward through the drill hole and well casing. The drilling mud serves primarily to lubricate and cool the drill bit, to flush away and raise the drill cuttings to the surface and to provide a temporary casing for the well hole. After passage of the mud through the well, the drill cuttings are removed and the drilling mud is again pumped into the drill pipe. Drilling muds consist essentially of dispersion of clays in water to which other adjuncts may be added to secure the desired colloidal characteristics in the mud. Starch products are added to improve the viscosity characteristics of the mud and to increase its water retention, that is, to lessen the tendency of the mud to lose water, particularly to the formation (50-58).

A satisfactory drilling mud should have sufficient fluidity to permit pumping at a proper rate without excessive power requirements. It should be sufficiently thixotropic to prevent the settling of sand and cuttings in the well when circulation is stopped temporarily and the mud should have sufficient density to support sand and cuttings within the well.

If a drilling mud which has a low water retention value is used when holes are drilled through ground formations such as sand or the soft, hydrous blue shale which is found in California, the mud loses water to the formation and the viscosity may increase tremendously. This not only increases the power requirements to circulate the mud but the mud may congeal or build up a cake on the formation which may impede or make impossible the removal of the drill from the hole. The addition of excess water in such cases, that is, the use of a less concentrated mud, results in a reduction in density and the mud is less suitable as a medium to carry away the drill cuttings. The addition of a hydrophilic colloid, such as a starch sol, is an effective remedy and although the starch product now used extensively does possess considerable viscosity in water, the addition of the starch to the mud serves to maintain the viscosity of the drilling fluid at a lower level than would be possible without the added starch.

In general, the more starch added to a drilling mud, the more is its water retention value increased. The greatest effect is observed when from 1 to about 3 pounds of starch are added per barrel of mud. Thereafter, as further quantities of starch are added, the percentage of gain in water retention value falls off sharply. Each drilling mud and each drilling operation requires a preliminary study to determine the proportion of starch required in order to maintain the water retention value at a satisfactory operating level. Another consideration, besides cost, limits the amount of starch which is added to a drilling mud. If small quantities of starch are added, the viscosity and gel or setting, properties of the mud are reduced, and these values reach a minimum in the range of 2 to 5 pounds of starch per barrel of mud. Further additions of starch cause some increase in viscosity and gel values.

The water retention of a mud is usually expressed as a water loss value. This is determined by placing a measured volume of the mud in a standard pressure filter and noting the volume of filtrate obtained through the filter paper over an interval of time at a given pressure. Another observation made in this test is the relative thickness of cake deposited on the filter during the interval of the test. This value is indicative of the tendency of the mud to build up a lining on the walls of the well.

The viscosity characteristics of a mud may be conveniently determined in the laboratory by use of the Stormer viscosimeter. In the field, viscosity is controlled by the use of the Marsh funnel. Another advantage for the use of a properly prepared starch product is that the plasticity of the mud is reduced as well as the viscosity. The result is that a relatively heavy mud containing starch and having a viscosity in the range 80 to 100 centipoises can be pumped with less difficulty than a thin suspension of clay in water with a viscosity in the range of 10 to 25 centipoises. Relative plasticity values may be determined from viscosity measurements in the Stormer viscosimeter at different rates of shear.

As in the application of starch in the clay-coating of paper, it is frequently desirable to add to the starch and clay mixture dispersing agents such as the pyro- and metaphosphates or alkali and other adjuncts such as fatty acids and fatty acid amides (59) which improve the colloidal properties of the clay for the use intended. Calcium ions, particularly from anhydrite ($CaSO_4$) tend to floc clays, and increase the viscosity and the water loss value. Dunn (60) recommended the addition of Na_2CO_3 and Gray (61) the addition of $BaCO_3$ to insolubilize the calcium.

One of the principal advantages starch has over several other water soluble colloids which could be used is that starch is less adversely affected by salt water or other contaminants encountered in some drilling operations. In some of the coastal areas, the use of fresh water in drilling may not be economically possible, and in other instances salt water may be encountered during the drilling at certain levels. Experience has shown (62) that muds saturated with respect to salt and unusable because of their high water loss value have been brought within operating specifications by the addition of 5 pounds of starch per barrel of mud. It was also found that the starch product was effective with muds contaminated by salts of magnesium and lime, acids, sulfides and cement.

Naturally, for the starch to be effective it should be gelatinized and dispersed into the mud. Since steam cooking equipment is not always available in oil fields, gelatinization by adding caustic alkali to the starch is used occasionally. However, to overcome the obvious disadvantage of preparing the starch dispersion on the drilling location and to secure the optimal colloidal properties in the starch, manufacturers have prepared special products for this use by modifying the starch, gelatinization, and drying. One such product is sold under the trade mark of Impermex (63, 64). Corn starch is admirably suited for use in this process and when the product is correctly made, it overcomes several of the otherwise undesirable characteristics or weaknesses of starch. In many drilling operations, it is necessary to use several thousand pounds' pressure to circulate the mud. Temperatures encountered are frequently in the neighborhood of 100°C. and often as high as 150°C. And, lastly, many operators prefer to use a highly alkaline mud with a pH value of about 12. All of these agents, pressure, heat and alkalinity tend to degrade starch sols and naturally when degradation becomes extensive, starch loses its desirable colloidal properties. Now a pregelatinized and roll-dried corn starch product redisperses with difficulty in water and its rate of dispersion in alkaline media is relatively low. This is due in part to reassociations which form when gelatinized starch is dried. On the other hand, the ability of the starch product to hydrate and its rate of hydration are at a satisfactorily high level. Therefore, the alkalinity of many drilling muds and the temperatures and pressure encountered during drilling operations disperse the starch product into very small, even submicroscopic, particles which are fairly well hydrated. These can exert their beneficial effects on the drilling mud mixture. However, the residual aggregates contain molecules which are sufficiently associated with each other so that the destruction of these molecules is very greatly retarded. Alkali presumably attacks the aldehydic ends of free starch chains and the action progresses along the chain until an associative block between chains (or a covalent bond leading to a side chain) is encountered, after which point the destructive action of alkali is materially retarded.

Naturally, if muds having a pH value closer to neutrality are used, other starch products more freely dispersible in water may be used with equal efficiency. However, the use of high alkalinity in muds, recommended by drilling mud manufacturers, has certain distinct advantages. The use of high alkalinity (*e.g.*, pH 12) is one of the cheapest and most effective means for preventing bacteriological activity which can be even more destructive in its action on the colloidal properties of the mud mixture than alkaline degradation. In addition, muds containing starch and having a high pH value can be contaminated with salt or large quantities of cement, and no significant change in the characteristics of the mud will result.

Therefore, by the addition of a corn starch product and alkali to a suitable mud base the cost for drilling mud may be greatly reduced and drilling operations may be simplified, since after the addition of the proper amount of starch product so as to reduce the water loss value to a satisfactory level and the adjustment of the pH value with NaOH, the mud may be used repeatedly throughout a drilling operation regardless of the conditions encountered in drilling. It is necessary thereafter only to make small daily additions of the starch product (to compensate for a minor fractional part lost by degradation) and an occasional addition of NaOH (probably not exceeding 4 to 5 oz. per barrel per day) to maintain the pH value.

Larsen (65) has patented the use of a pregelatinized tapioca starch, preoxidized by boiling with 0.7% by weight of BaO_2. Cannon (66) has found that chlorinated starch is relatively resistant to fermentation. Common experience is, however, that oxidized starches are less stable in colloidal properties at high pH and high temperatures than the corresponding starches from which they are derived. Cannon (67) has patented the use of a starch esterified with an aliphatic acid of low molecular weight. Kerr and Schink (68) have shown that the branched polymer fraction of starch is extremely effective in reducing water loss and recommend the use of a pregelatinized waxy starch.

In summation it may be said that the use of starch products in oil well drilling muds in recent years has been a major development in this field, since it has permitted drilling into formations which it would not have been profitable to do without starch, and it has resulted in greatly reduced drilling costs in normal operations.

The use of a starch product in the cutting of cores from formations has greatly facilitated the procurement of representative specimens from which reliable analytical data may be obtained. Cores are usually taken of formations believed to contain oil or gas in order to evaluate oil field properties for the purpose of commercial exploitation. In the case of fields under development, it may be desirable to estimate reserves and in the case of partially depleted fields, accurate data on the productive zone is required when further, and frequently more economical, production is contemplated by repressuring or when secondary recovery by flooding with water is planned. A small error in the calculation of the available oil from analytical data on specimen cores, caused by failure to obtain representative samples of the formation, may result in failure rather than commercial success for the operation.

Core holes are usually drilled by special bits and pressure core barrels have been used for taking cores in a manner which eliminates many of the mechanical obstacles to the procurement of representative samples. However, a serious error may be caused by the flushing action of the drilling fluid. As this passes vertically downward from the bit, the fluids in the stratum being cored may be washed out by the mud to an extent depending on its permeability, rate of penetration and the magnitude of the hydrostatic pressure of the mud in excess of the pressure in the formation. Experience has demonstrated that by the use of an alkaline mud and the addition of a sufficient amount of pregelatinized corn starch product to maintain the water loss value at a minimum, cores have been obtained which have proven to be very much more representative of the formation than when only mud and water were used in the core drilling operation.

Frequently oil wells are cased with Portland cement. This is ordinarily accomplished by flushing down the sides of the drilled hole with a slurry of sand and cement in water at a suitable consistency. If it is too thin, the casing may be weak due to a penetration of water and cement into the formation; if it is too thick, the cement casing is difficult to pump, may deposit an excessively thick wall on the hole and may set before the cement is in place. In any event, a slightly retarded setting rate is desirable in order to allow proper disposition of the cement. Therefore, several of the problems encountered with the use of drilling fluids are also encountered with the employment of cement casings plus, in addition, the problem of properly controlling the setting rate, occasionally under adverse conditions such as elevated temperatures. Properly modified starch has both the property of increasing the water retention value of thin cement slurries and that of retarding the setting rate of the cement. Solution of the problems involved is complicated by the fact that native starch does not have quite the water retention value per unit weight desired for the purpose, and the addition of sufficient starch increases the viscosity of the cement mixture and retards the setting rate to an undesirable extent. Hydrolysis of the starch before use increases the water retention value at first and subsequently causes a decrease as the molecular units become smaller. However, hydrolytic scission of starch molecules increases the effectiveness of the product as a retarder for the setting of cement and if an amount of acid-modified starch is added which is just sufficient to retard the setting rate to a desirable extent, then the water retention value will not have been increased as much as is customarily required.

Other problems arise when a pregelatinized starch product is used in order to avoid the necessity for gelatinizating the starch on the oil well location. Pregelatinized roll-dried products do not disperse readily in cement mixes at low temperature. Although the dispersion may be satisfactory when the cement reaches temperatures of 75°C. and higher, which are frequently encountered in some wells, it is desirable that the starch become at once effective, particularly in respect to its retarding effect on the setting rate of the cement. The problems involved have been partially solved in several ways. Among these are the use of mixtures consisting of a cold water soluble colloid of high molecular weight (which will be immediately effective at lower temperatures) and a starch product. Another is the use of dextrins (69) which are partially cold water-soluble. The use of oxidized and acid-modified starches (70) has also been claimed, which are soluble to the extent of about 65% in water at 80°F. so that a part of the starch, at least, will become immediately effective in retarding the setting rate of the cement. An inspection of the problem by the writer indicates, as in the study of drilling muds (68) that the branched polymer fraction of starch is inherently better for this use than the linear polymer or than a mixture of the two types and that a suitable adjunct for cement is best made by a proper modification of the branched polymer of starch or starches which exhibit little or no linear polymer characteristics.

b. Refining Aluminum Ores. An important use for starch established within the last decade in the field of mining and metallurgy is that of improving the recovery of aluminum from bauxite ores. The advantage of starch is in its ability to coagulate and depress iron oxides and other impurities in alkaline digests of the ore. Indeed, actual stabilization of the sodium aluminate by the starch is reported in the patent issued to Brown (71).

Depending on its composition, the ore is usually digested at about 145°C. with sufficient NaOH so as to produce a liquor containing about 90 g. of Al_2O_3 per liter, as sodium aluminate. The digest is blown to a tank where the insoluble gangue, or red mud as it is often called, is separated. The aluminum is in an unstable state and will precipitate almost quantitatively as an aluminum hydrate on standing, particularly at lower temperatures. Purification of the digest is done, therefore, at about 125°C. The temperature and alkalinity of the digest makes filtration difficult to accomplish on a large scale in an economical manner. Accordingly, sedimentation is the preferred procedure. The difficulty in the past with sedimentation has been, except with high grade ores, that of the time required for the red mud to settle both in the initial separation from the ore digest and from washing the mud to recover the aluminate in this slimy mass. With low grade ores, sedimentation methods have given low aluminum recovery yields since long sedimentation times have permitted the aluminum to precipitate as the hydrate with the red mud. Moreover, the necessity for providing sedimentation tanks having sufficient capacity has made the process unattractive.

However, when a small amount of starch, of the order of 0.01 to 0.2 g., is added (per liter of digest), the sedimentation rate of the red mud is greatly increased and the sodium aluminate may be stabilized in solution at this stage of the process. The amount of starch product added will depend upon the type of starch used and the amount of red mud to be precipitated. No more starch should be added than can be carried down and collected with the red mud for if an appreciable excess remains after sedimentation of the mud, this excess may tend to prevent separation of the aluminum hydrate in subsequent stages of the process. Since starch suffers a chemical degradation in alkali at high temperature with a simultaneous loss in colloidal properties, it would seem wise to add the starch in a mixing tank after the ore digesting procedure and to use a starch product having a low alkali lability value. The writer found that for some unexplainable reason starch and protein mixtures, such as corn starch and gluten in the proportion at which they are obtained in wet-milling before the table separation step, are more effective per unit weight in increasing the sedimentation rate of the red mud than an equal weight of starch separated from these naturally occurring mixtures. Starches, the stability of which has been increased against alkaline degradation, such as, for example, the treatment of starch with formaldehyde or sodium stannate, are measurably more efficient than untreated starch.

In this application the starch is dissolved and is, very likely, molecularly dispersed. The effect observed is, therefore, that of the molecular units of starch. Although starch solutions, particularly those of the branched polymer fraction, have the ability to depress iron oxide and other insoluble material of this character, a smaller effect, if any, is observed on oxides of less electropositive elements such as silica. Indeed, suspended silica may be stabilized under certain conditions. A limitation to the use of starch in improving separations of alumina from low grade ore is the silica content of the ore. Ordinarily ores containing as high as 5 to 6% SiO_2 can effectively be handled by the processes indicated above. However, in using the lowest grade ores which contain large amounts of silica or unusual amounts of organic matter or constituents not precipitated by starch, other adjuncts must be used in order to carry out the sedimentation of the gangue economically.

c. *Flotation of Ores.* Starch has long been recognized as a depressant for certain constituents of finely divided ores in flotation processes used to collect and concentrate the desired constituent (72). Starch appears to be most effective when used as a depressant for nonsulfide ores and particularly metallic oxides. Of these, the concentration of iron oxides from low grade ores such as hematite has been extensively studied in recent years. In view of the tremendous and ever growing demand for iron and the dwindling supplies of high grade ore in this country, attention has been directed, of necessity, to the study of ways and means economically to use lower grade ores and tailings previously discarded in years past from the processing of high grade ore. Indeed, attention has been focused on this problem to such an extent in recent years that already some states have considered legislation requiring the processing of a definite proportion of low grade ore with other ores of higher grade processed. Should the United States be required to rely for its requirements for iron on low grade ores in future years in a proportion now indicated, and should starch prove to be as efficient and economical to use as an adjunct in flotation processes, as present research and preliminary operations indicate, then this demand for starch would present a staggering problem to the starch industry. The estimated volume for this new outlet would exceed any of the present ones, and would, it appears, exceed in several decades the present total starch producing capacity of all wet millers in this country. These considerations are an effective answer to those who propose that the market for starch products has been saturated, that potential markets have been exhausted and that the utilization of starch is on the threshold of decadence.

Clemmer and co-workers (73) have reviewed the principles of flotation as applied to the processing of iron ores. The process consists essentially of grinding the ore to a very fine state of subdivision (through 100 mesh), the addition of water to form a slurry having about 10 to 30% solids, the incorporation of frothing agents and conditioners and the addition of depressants followed by separation of the ore constituents in suitable flotation equipment.

A review of the patent literature discloses that many types of frothing agents have been used with hematite. Where the gangue consists principally of silica and it is desired to float the gangue and collect the iron oxide by settling, aliphatic amines, higher fatty acids, soaps, resin acids and the like are apparently quite effective. Brown and Tartaron (74) outline such a process involving the use of oxidized starch as a depressant. The ore, containing 22% iron oxide, is ground to pass 100 mesh and is made into a slurry with water at a concentration of 10% solids. Oxidized starch cooked in a 2% solution with water is added in a ratio of 2.5 pounds of starch per ton of iron ore. Four pounds of lime, in a 25% suspension, and a mixture of 0.95 pounds of fatty acids and 0.5 pounds of cresylic acid are added. After a frothing treatment and flotation of the gangue, the iron ore concentrate was found to contain 60% iron oxide which represented about 80% recovery of the iron. Oxidation of the starch to the "dextrin" stage with permanganate, hypochlorite or peroxide is recommended by these workers.

These investigators also suggested (75) the use of starch solubilized by boiling in 10% NaOH solution in a ratio of 1.5 pounds of starch per ton of ore. Later, however, Brown and Tartaron recommended (76) a pretreatment of the starch with acid. The starch is partially hydrolyzed by boiling a 2% suspension for one minute at a pH value of 1.6 to 2.2. In this case, about 2.5 pounds of starch per ton of ore is used. Comparable results were obtained with thin boiling starches such as Linit brand starch, that is, starches pretreated with acid at temperatures below the gelatinization point, by starch manufacturers.

Clemmer and Clemmons (77) recommended grinding the iron ore with lime, floating the SiO_2 with anionic collectors and using starch or dextrin as dispersants.

Studies by the author have shown that the branched polymer fraction of starch is particularly effective in depressing iron oxide in processes similar to the one above and that the linear polymer, in comparison, is almost without effect. However, it is also apparent that a certain range of molecular size for the branched polymer is desirable for the best results. For example, the native branched polymer of corn starch is not as effective as one which has been partially degraded. Molecular weight ranges corresponding to DP values between 50 and 250 appeared to give the most efficient results.

Probably the simplest explanation for the effect of these dissolved molecules is that when a limited amount of starch sol is added, it is preferentially adsorbed by the metallic mineral particles rather than the silica. Thus, a hydrophilic layer is formed around the iron particles, displacing the air, permitting them to become wetted and to sink. Then, when an agent such as an aliphatic amine is added, this would be taken up by the silica through the amine groups. The aliphatic groups projecting from these particles become attached to air bubbles when a foaming agent is added and the silica is thereby caused to float and is removed from the ore.

With an expansion in the application of flotation to the refining of various types of ore, many other potential uses for starch products have developed. For example, in recent years flotation has been applied to the separation of NaCl from KCl (sylvite) in salt deposits (sylvinite) found in New Mexico. A saturated brine is used as the flotation medium. A modified starch product may be added in a preliminary or conditioning treatment wherein impurities are removed as a floatable gangue. The salts after further grinding are then separated from each other. In one process (78), KCl is floated from NaCl using starch as a depressant. The slyvinate ore is crushed and deslimed. After grinding to a suitable mesh size, the ore is mixed with a saturated solution of the ore, an aliphatic amine, such as lauryl amine acetate a frothing agent, such as pine oil, and starch. When 1 pound of starch, boiled in

0.5% NaOH, is added per ton of ore in a froth-flotation treatment, the recovery of the KCl is raised from about 80 to 90% in the primary concentrate. In addition, the KCl lost ordinarily in the middlings is reduced by some 50% of the loss usually experienced.

According to a patent issued to Eigeless (79), dextrin treated with sodium silicate may be used to depress calcite in certain ore pulps.

d. Sedimenting Agent. Starch products have been used to some extent as sedimenting agents in applications which are probably quite different in fundamental principles from those of depressants in flotation. Clarification of process waters in cases where disposal is impractical or where re-use is desirable or where recovery of suspended solids is important economically can be accomplished by the addition of relatively small amounts of pregelatinized starch. These are usually substantially unmodified starches, partially pasted and dried, as, for example, those made by passing a starch filter cake or starch slurry through heated rolls. The product ground to a fine powder rehydrates in water but does not dissolve or redisperse, except to a limited extent. These relatively large colloidal units settle out and carry with them finely divided and suspended particles. A relatively important example is in the mining of anthracite coal. Many mine operators wash the coal and pass the wash water to holding tanks in order to recover the fine coal dust and to re-use the water in the next washing operation. The addition of pregelatinized starch products in small amounts, depending on the amount of dust carried with the wash water, materially speeds up the sedimentation rate of the suspended material.

e. Binding Agent for Ores. In some ore refining operations, the ore at some stage is reduced to a very fine powder. This is particularly true in cases where refining involves flotation, such as the treatment of hematite, described above. In order to treat the enriched ore powder in the conventional types of furnaces, it may be necessary to form aggregates or lumps from this powder so that it is not blown away by convection currents. One method is to form briquettes from the powdered ore. Modified starch products, particularly dextrins, are used as binding agents in these processes.

Starch products have also been used in forming briquettes from fine coal particles (80). Where the briquetted coal is intended for home use and may be burned in an open fireplace, a problem involved is the tendency of starch to form products of combustion which have an unpleasant odor, particularly when there is an insufficiency of available oxygen. The product is, of course, suitable for combustion in furnaces with proper draught and where these are equipped with grates for burning lumps, briquette formation makes possible the utilization of a lower grade of coal.

j. Foundry Operations. In terms of volume sold, the most important outlet for starch products in the past in the field of metallurgy has been for use as a binding agent in the production of cores from sand in foundry work. It has been estimated (81) that nearly two hundred million pounds of starch products have been used annually for this purpose in recent years. Molds are made to form the sand cores and these are filled with sand which has been moistened with water containing a suitable binding agent. After compression in the mold, the wet core is removed and given a baking treatment. Thereafter the cores are used to cast hollow shapes by pouring molten metal around them. After the metal cools, the sand core is removed from openings provided in the cast.

A suitable binding agent should disperse or rehydrate in cold water, blend evenly with the sand and absorb a large share of the water added so that the wet sand will set into shape without undue delay. At this point, the adhesive should have developed sufficient strength so that the sand core may be removed from the mold intact and handled during the baking operation. This is called "green bond strength" in practice. After baking, sufficient strength should develop to permit transference of the core, possibly to storage racks, or directly to the metal casting units and to retain its strength during pouring of the metal. Thereafter the binder should burn out evenly permitting the sand to be poured from the cast after the metal has solidified. In burning, the binder should not create an undue amount of gases which might distort the cast before solidification or create blow-holes, if these gases are trapped locally.

In ferrous metallurgy, a starch product made by passing a filter cake of corn starch and gluten between heated rolls is a very suitable binder for sand cores in many operations. Except for being partially gelatinized, the starch is essentially unmodified. One such product is sold under the trade mark of Mogul (82-84). For certain other applications, a small proportion of dextrin is incorporated in these products. Thus, the green bond strength is improved in a product which may be admirably suited in respect to other physical properties but which may be weak in this characteristic. A British patent (85) outlines the following manufacturing procedure not only for increasing the green bond strength by pretreatment of starch and protein mixtures with acid but for perfecting the binder in other respects by the addition of a mixture of oils. Four hundred gallons of corn starch, preferably the "squeegee" obtained in tabling which contains about 11.5% protein, is made into a slurry with water so as to contain 33% solids. To this is added 4.5 gallons of HCl sufficient to make the concentration 0.56% in respect to the acid. The mixture is held for 17 hours at 50°C. During this interval the fluidity reaches a value of 40 to 50 (as measured by and calculated from the Scott test for hot paste viscosity). Soda ash is then added to raise the pH value to the range 6.0 to 6.8. The slurry is then gelatinized by raising the temperature to from 80 to 90°C. and the paste is drum-dried. After the dried product is ground to pass No. 30 mesh sieves, 85 parts of the starch product is blended with 15 parts of borax and 50 parts of core oil. The core oil is a mixture of 50 parts linseed oil, 30 parts rosin oil and 20 parts of Mexican bitumen oil. The recommendations are to use the mixed binder in a ratio of 2% based on the weight of dry sand.

Roll and Riess (86) have compared the behavior of various binders such as starch products and oils which have been used as core binders. Core binders are also made from starch products by dry millers who pretreat flours for this use. Mehltretter (81) has shown that satisfactory binders can be made from corn, wheat, and rye starches and their flours. Heat gelatinized barley starch and flour, however, gave cores with satisfactory green strength, but low baked strength.

g. Electroplating of Metals. The use of starch products, such as the metal starchates, in electroplating has been patented by Gaver (87). Sodium starchate, wherein the sodium is attached to the second carbon in the glucose residues, is prepared by reacting the alkali with starch in an alcoholic medium. The sodium may then be replaced by heavy metals such as nickel.

A plating bath for the electrodisposition of metal consists of an aqueous solution providing ions of the metal to be deposited by electrolysis and also a colloidal solution of the metal as a starchate. The starchate is claimed to facilitate the production of bright, smooth metal coatings on electrolysis. Gaver gives an illustrative formula suitable for nickel plating consisting of the following parts by weight: Nickel sulfate, 135-165; nickel chloride, 20-30; boric acid, 20-40; and nickel starchate, 5-25. Sodium starchate may be used instead of the nickel starchate and the pH of the bath adjusted to the desired value by adding H_2SO_4.

h. Coatings for Metals. Haage (88) has patented the use of coatings on sheet iron which consists of solutions of either dextrose or starch in order to prevent the sticking of the iron sheets when heat treated. Wachter (89, 90) claims a process for preparing coatings from starch to be applied to metal surfaces in order to inhibit corrosion. An aqueous sol is prepared by treating 40-85 parts of dextrin with 0.02 to 10 parts of $NaNO_2$ and adding a polyhydric alcohol, such as glycerol, as a plasticizer. Modified starch may be used and other plasticizers insoluble in hydrocarbons may be used. These coatings are applied as linings to metal tanks or drums used to hold gasoline and other hydrocarbons which contain only minor amounts of water.

8. Plastics and Coatings. Formerly the use of starch in plastics, films, fibers and coatings was limited principally to the use of derivatives such as esters and ethers. The preparation, properties and use of these derivatives for such purposes have been reviewed by Degering (91), Mullen and Pacsu (92), Whistler and Hilbert (93), Radley (94) and others.

The reason that these derivatives have not obtained a wider application in this field is that, as a class, their cost of production is not significantly lower than competitive products to be attractive, particularly in view of the fact that the products appear to possess no outstanding merits to warrant a preference for their use. This is likewise true of the use of nitrostarches in the compounding of lacquers. The latter subject has been reviewed by Kraus (95).

More recently different types of derivatives have been extensively described and used. In these the starch or its derivatives enters into a copolymerization reaction. There are two general types, one in which the starch is first etherified with an unsaturated hydrocarbon such as a vinyl or allyl group and this unit, in a sense, becomes the monomer for the polymerization and the other where starch is caused to react with amine-aldehyde units which may have already been partially polymerized. The chemistry of the copolymerization of polyhydric alcohols, such as the carbohydates and vinyl groups, has been described by Reppe (96). The reaction is induced by forming the alcoholate, such as sodium starch, and treating this under pressure with acetylene. The acetylene reacts with the alcoholate group (or the alcohol in the presence of NaOH or KOH) to form a vinyl group attached through an ether oxygen to a carbon on the carbohydrate chain. Primary alcohol groups react readily and the acetylene may be diluted with an inert gas such as nitrogen.

The allyl starch products have received far greater publicity in America and more is known of their use and methods for application, particularly in protective coatings. Inasmuch as allyl bromide or chloride is commonly used as the etherifying agent, costs have been quite high. However, the product when dissolved in solvents commonly used for paints and varnishes and applied as a coating, develops a hard smooth surface which is extremely resistant to solvents, acids and alkalis (97, 98). Thus, superior lacquers, varnishes, cements and impregnating compounds are produced. Coatings are reported to be transparent, possess high gloss, hardness and adequate flexibility. During curing of the coating after application of the film, polymerization of the allyl starch is completed. Inasmuch as the allyl starch most suited for coating work must be controlled both in respect to viscosity and degree of substitution of the original starch, and inasmuch as improvements are or have been made in controlling these properties which involve reductions in the amount of allyl halide used, costs for making this starch derivative have been steadily reduced until the starch product is now in a fairly favorable competitive position.

The second type of polymerization involving starch which has been useful in plastic applications is illustrated by the following patents. Ericks and Whitaker (99) claim a condensation product of guanidine and starch which is prepared by heating tapioca starch with guanidine carbonate in the presence of HCl. Hansen (100) has patented a starch-melamine molding composition. This is prepared by mixing 25 parts of a melamine-formaldehyde resin with 75 parts of starch and 15 parts of water, adjusting the pH to 7-11 and milling the mixture. This can be molded to produce a plastic material having great flexural strength. Van Linge (101) has patented a molding powder which is made by mixing 1,000 parts of potato starch, 600 parts of 40% formalin and 3.5 parts of lactic acid. After 30 minutes, 160 parts of urea and 200 parts of water are added and the mass is heated in a closed vessel until the starch is gelatinized. The mass is then dried and ground. The powder is suitable as a press powder. A patent was granted to Van Linge (102), for a molding powder made from a mixture of starch, aldehyde and a component able to form a resin. The mixture is heated to gelatinize the starch, then ground and dried. An acid may be added to promote the polymerization. The powder is pressed into shape with heating.

Some miscellaneous types of plastic materials made from starch are as follows: Hall (103) has disclosed a free-flowing puncture sealing composition comprising a cold water swelling starch, water, a polyhydric alcohol, fibrous material, and granulated rubber. Perbohner (104) has patented an artificial timber veneer made by mixing ground wood with $MgCO_3$, water and one or more of the following substances: resins, gums, starch, vegetable oils, and rubber latex. The mixture is then dried and pressed with or without the application of heat to form the desired products. Starch may be converted into material suitable for use as a filler in plastics or as a component for opaque coatings by heating the starch for 2 hours at 200°F. with 1 to 15% formaldehyde and 0.1% HCl, followed by cooling and neutralization (105). Seberlich (106) has reviewed the subject of the use of starch in film forming compositions and plastics.

9. **Printing.** A substantial outlet for starch exists in the printing field. The largest use here is the use of dry, powdered starch as an adjunct in printing inks. Mobile corn starches are employed for this use and give superior results when compared with other starches. Experimentally, any type of pretreatment which induces mobility in the starch when finished as a dry powder such as those methods given in an earlier chapter, are satisfactory.

Amick (107) has recently patented the application of carbohydrates in vehicles used for planographic printing. In this, the carbohydrate is essentially a bodying ingredient and is reacted with acid amides such as those of carbonic, thiocarbonic and cyanuric acids. The mass is used to incorporate a printing color which is capable of being directly fixed on cellulose without mordanting. When the composition of this vehicle is 45 to 65% carbohydrate and 6 to 25% amide, it is a thermoplastic putty-like mass which is hard at room temperature and displays plasticity at higher temperatures.

Starch and starch products are used in the making of offset printing forms (108). In the production of a hydrophilic film on aluminum or zinc, a 1% starch or 40% dextrin solution has been found equally effective. The film can be removed from these metal surfaces with water.

10. **Water Treatment.** Starch products may be used in treatment of hardness of waters fed to boilers, in the clarification of water supplies for industrial use, and in the recovery of suspended solids in industrial wastes. Representative examples for these uses are to be found in the following patents.

Sherwood (109) has patented a compound consisting of 50 parts of starch, such as tapioca, 45 parts of NaOH (40-45% solution) mixed together rapidly and to which is then added 100 parts of anhydrous Na_2CO_3. Binding agents such as Na_2HPO_4, tannin, and lignin derivatives may also be added. The compound may be fed directly to steam boilers or may be used with other chemicals employed in the treatment of water.

Muddy waters may be clarified by the addition of a paste made of 20 parts of a starch product and 50 parts of $FeSO_4$ per million parts of water. The starch product which accelerates the sedimentation rate of the suspended mud is made by passing a starch, such as potato, with 1% $NaCO_3$ over heated rolls and grinding to a powder (110).

The clarification of industrial waste waters and the recovery of the suspended solids may be accomplished by the addition of powdered pregelatinized starch products. A suitable product may also be made in the form of a gel by treating starch at 70°C. with concentrated solutions of metallic chlorides, such as zinc, calcium, magnesium, or lithium until a gelatinized mass is obtained (111).

BIBLIOGRAPHY

1 Wilson, R. E., and Bunnell, L. A., An Investigation of the Use of Starch in Vitreous Whiteware Body. Thesis, New York State College of Ceramics, Alfred University (1947).
2. Ellis-Anwyl, R. H., *Brick & Clay Record,* **108**, No. 1, 54 (1946).
3. Hoggatt, G. H., U.S. Patent 2,388,543 (1945).
4. Anon., *Drug & Cosmetic Ind.,* 444 (March, 1946).
5. Classen, A., German Patent 92,259 (1896).
6. Classen, A., German Patent 94,282 (1896).
7. Herstein, B., U.S. Patent 982,673 (1911).
8. Pierson, G. G., U.S. Patent 2,417,611 (1947).
9. MacMasters, M. M., and Blom, R. H., *Chemurgic Digest,* No. 37, (Dec., 1945).
10. Ling, A. R., and Nanji, D. R., *J. Chem. Soc.,* **123**, 2666 (1923).
11. Bice, C. W., MacMasters, M. M., and Hilbert, G. E., *Science,* **100**, 227 (1944).
12. Bice, C. W., and MacMasters, M. M., U.S. Patent 2,423,475 (1947).
13. Spengler, H., and Jud, J., *Pharm. Acta Helv.,* **18**, 565 (1943).
14. Karrer, P., Canadian Patent 428,839 (1945).
15. Walter, M., Canadian Patent 428,564 (1945).
16. Husemann, E., Kaulla, E. N., v., and Kappesser, R., *Z. Naturfoch.,* **1**, 584 (1946).
17. Kerr, R. W., and Severson, G. M., *J. Am. Chem. Soc.,* **65**, 193 (1943).
18. Minto, W. L., U.S. Patent 2,383,334 (1945).
19. Termansen, J. B., *Farm. Tid.,* 53, 755 and 773 (1943).
20. Khesin, M. J., *Farmatsiya,* **13** (1941).
21. Borglin, J. N., U.S. Patent 2,392,359 (1946).
22. Brown, K. R., U. S. Patents 2,357,077-8 (1944).
23. Gaver, K. M., U. S. Patent 2,397,732 (1946).
24. Höppler, F., *Chem. Ztg.,* **67**, 72 (1943).
25. Nieuwenhuis, K. L., *Chem. Weekblad,* **43**, 510 (1947); *J. Am. Oil Chemists' Soc.,* **25**, 31 (1948).
26. Nusslein, J., and Pauser, K., U.S. Patent 2,335,194 (1944); *J. Textile Inst.,* **35**, A354 (1944).
27. Petersen, B., U.S. Patent 2,422,633 (1947).
28. Gaver, K. M., U.S. Patent 2,347,678 (1944).
29. Gaver, K. M., U. S. Patent 2,347,679 (1944).
30. Gaver, K. M., U.S. Patent 2,347,680 (1944).
31. Groen, M. G., and Krizkovsky, F. L. P., Dutch Patent 58,868 (1947).
32. Ashford, W. R., Evans, T. H., and Hilbert, H., *Can. J. Research,* **24B**, 246 (1946).
33. Radley, J. A., *Mfg. Chemist,* **17**, No. 3, 83 (1947).
34. Berl, E., and Kunz, W. C., *Ann.,* **520**, 270 (1935).
35. Nobel, A., German Patent 57,711.
36. Sadler, S. S., U.S. Patent 1,211,761 (1917).
37. Helle, J., and Kunz, W. C., U.S. Patent 2,127,360 (1938).
38. Caesar, G. V., and Goldfrank, M., *J. Am. Chem. Soc.,* **68**, 372 (1946).
39. Snelling, W. O., U.S. Patent 2,333,275 (1943).
40. Snelling, W. O., U.S. Patent 2,371,000 (1945).
41. Carey, C. H., U.S. Patent 2,333,637 (1943).
42. E. I. duPont de Nemours and Co., and Davis, C. O., British Patent 564,607 (1944).
43. Gaver, K. M., U.S. Patent 2,389,771 (1945).

44. Reschke, C., German Patent 717,273 (1942).
45. Reschke, C., German Patent 726,979 (1942).
46. Anon., Liquid Starch, *Chem. Inds.*, **61**, 613 (1947).
47. Spilka, S., U.S. Patent 2,228,784 (1941).
48. Pecker, J. S., Pecker, E. A., and Pecker, A. G., U. S. Patent 2,424,050 (1947).
49. Peters, A. F., U.S. Patent 2,435,901 (1948).
50. Perkins, F., and Craft, B. C., *Petroleum Technol.* (Jan., 1943).
51. Perkins, F., and Craft, B. C., *Am. Inst. Mining Met., Engrs.*, Tech. Pub No. 1551 (1943).
52. Sawdon, W. A., *Petroleum Engr.*, **14**, 115 (July, 1943).
53. Bond, D. C., U.S. Patent 2,357,497 (1944).
54. Vietti, W. V., U.S. Patent 2,357,565 (1944).
55. Bond, D. C., U.S. Patent 2,360,544 (1944).
56. Foster, J. L., U.S. Patent 2,364,434 (1944).
57. Gray, G. R., U.S. Patent 2,369,560 (1944).
58. Radford, H. E., *Petroleum Engr.*, **18**, No. 9, 72 (1947).
59. Booth, R. B., U.S. Patent 2,375,616 (1945).
60. Dunn, T. H., U. S. Patent 2,391,622 (1945).
61. Gray, G. R., *Petroleum Engr.*, **18**, No. 9, 142 (1947).
62. Anon., Drilling Mud, Baroid Sales Div., National Lead Co., Los Angeles, 11, No. 1 (1944).
63. Sawdon, W. A., *Petroleum Engr.*, **16**, No. 3, 67 (1944).
64. Sawdon, W. A., *Petroleum Engr.*, **17**, No 4, 92 (1945).
65. Larsen, D. H., U. S. Patent 2,417,307 (1947).
66. Cannon, G. E., *Oil & Gas J.*, **45**, No. 52, 101 (1947).
67. Cannon, G. E., U.S. Patent 2,417,235 (1947).
68. Kerr, R. W., and Schink, N. F., *Paper Trade J.*, **120**, 86 (TAPPI) (1945).
69. Swayze, M. A., U.S. Patent 2,374,628 (1945).
70. Andes, R. V., and Ludwig, N. C., U.S. Patent 2,429,211 (1947).
71. Brown, R. W., U.S. Patent 2,280,998 (1942).
72. Weinig, A. J., and Carpenter, C. B., The Trend of Flotation, 3rd Ed. *Quart. Colo School Mines,* **32**, No. 4, pp. 20 and 61 (1937).
73. Clemmer, J. B., Clemmons, B. H., Rampacek, C., Williams, M. F., and Stacy, R. H., Bur. of Mines Report of Investigations, No. 3799 (1945).
74. Brown, E. H., and Tartaron, F. X., U.S. Patent 2,364,618 (1944).
75. Brown, E. H., and Tartaron, F. X., U. S. Patent 2,364,777 (1944).
76. Brown, E. H., and Tartaron, F. X., U.S. Patent 2,364,778 (1944).
77. Clemmer, J. B., and Clemmons, B. H., U.S. Patent 2,383,467 (1945).
78. Tartaron, F. X., Cole, A. T., and Duke, J. B., U. S. Patent 2,288,497 (1942).
79. Eigeless, M. A., Russian Patent 58,390 (1940).
80. Erickson, J. A., U.S. Patent 2,349,342 (1944).
81. Mehltretter, C. L., Cereal Core Binders, *Foundry*, **75** (Dec., 1947).
82. Gieseke, F. O., U. S. Patent 1,939,973 (1933).
83. Gieseke, F. O., U.S. Patent 1,943,382 (1934).
84. Gieseke, F. O., U. S. Patent 1,974,915 (1934).
85. Nicolson, J., British Patent 543,202 (1942).
86. Roll, F., and Riess, A., *Giesserei*, **29**, 320 (1942).
87. Gaver, K. M., U.S. Patent 2,406,072 (1946).
88. Haage, H., German Patent 716,962 (1942).

89. Wachter, A., U. S. Patent 2,357,275 (1944).
90. Wachter, A., U. S. Patent 2,357,276 (1944).
91. Degering, E. F., in Chemistry and Industry of Starch, Academic Press, New York, 187 (1944).
92. Mullen, J. W., and Pacsu, E., *Ind. Eng. Chem.*, **35**, 381 (1943).
93. Whistler, R. L., and Hilbert, G. E., *Ind. Eng. Chem.*, **36**, 796 (1944).
94. Radley, J. A., *Mfg. Chemist*, **17**, No 3, 83 (1947).
95. Kraus, A., *Z. ges. Schiess-u. Sprengstoffw. Nitrocellulose*, **38**, 170 (1943).
96. Reppe, Advances in Acetylene Chemistry. An address before the Technical Society in Harnach-House, Berlin-Dahlem, July 26, 1940.
97. Nichols, P. L., and Hamilton, R. M., *Oil Paint and Drug Reptr.*, **146**, 46 (1944).
98. Nichols, P. L., Hamilton, R. M., Smith, L. T., and Yanovsky, E., *Ind. Eng. Chem.*, **37**, 201 (1945).
99. Ericks, W. P., and Whitaker, M. C., U. S. Patent 2,389,723 (1945).
100. Hansen, D. W., U. S. Patent 2,408,065 (1946).
101. Van Linge, R. A., Dutch Patent 56,721 (1944).
102. Van Linge, R. A., Dutch Patent 57,967 (1946).
103. Hall, L., U. S. Patent 2,357,650 (1944).
104. Perbohner, S., British Patent 560,929 (1944).
105. Pierson, G. G., U. S. Patent 2,417,611 (1947).
106. Serberlich, J., *Modern Plastics*, **18**, 64 and 98 (1941).
107. Amick, C. A., U. S. Patent 2,404,769 (1946).
108. Sineguf-Lavremko, A., *Chem. Centr.*, **II**, 1685 (1941).
109. Sherwood, F. E., U. S. Patent 2,376,885 (1945).
110. Scholten's Chemische Fabrieken, Dutch Patent 51,552 (1941).
111. Samuel, J. O., British Patent 516,294 (1939).

Chapter XXIV

METHODS OF ANALYSIS FOR STARCH

Analyses with which chemists are concerned in their study of starch and modified starch products may be grouped under three headings, first, a determination of the amount of starch in products or in sources, such as grains, secondly, the purity of the starch in respect to the carbohydrate when it has been separated, and thirdly, the extent to which the starch may have been altered, either intentionally or otherwise, by chemical or physical processes.

Ordinarily, a product is given a microscopical examination by the starch chemist and quite often much can be learned, using the information outlined in Chapter I, by staining and heating procedures as to whether the product contains starch, the type of starch it is and approximately how much starch is present in the sample. Of course, products in which the starch is not in the form of granules offer special problems in identification. Some of these are also discussed in Chapter I. Having made the preliminary examination, procedures for obtaining the desired analytical information may be selected accordingly.

1. Quantitative Determination of Starch. The procedure selected for the determination of starch will depend on the nature of other substances present in the mixture which may interfere in some methods and not in others, on the chemical state of the starch and on the relative amount present. No attempt will be made to list all of the procedures which have been used or proposed. It is believed that the following methods which are outlined will provide a sufficient basis for determination in the majority of cases. Special products may require additional study in order to adjust these methods to the problem at hand, or may make it necessary to devise new methods. New uses are continually being developed for starch and accordingly the number of special products containing starch is constantly increasing and particular methods for their analysis cannot be anticipated. Procedures which have been most useful in the past have been based on hydrolysis of the starch by acid and the determination of glucose, diastatic hydrolysis, solution of the starch by a suitable solvent and optical rotation readings on the solution and, solution of the starch followed by iodine spectrophotometry.

a. Acid hydrolysis. The product is ground to a powder so as to pass about a 40 mesh sieve, or if this is impracticable, the sample may be wet-ground in a Waring Blendor with about 200 ml. of water. In either case, a weighed quantity of sample, representing about 2.5 g. of dried materials such as cereals products and potatoes, is stirred or shaken with 250 ml. of water at room temperature for an hour. The insoluble residue is transferred quantitatively to a small filter with the aid of an additional 250 ml. of water, then taken up in 200 ml. of 0.5 *N* HCl and boiled under reflux for 2.5 hours. The solution is cooled to 20°C., adjusted to pH 5 with NaOH, and the final volume made up to 250 ml. This solution is filtered or centrifuged and the glucose equivalent in an aliquot is determined by suitable procedures.

Ordinarily, a copper reagent is satisfactory and glucose may be estimated by the gravimetric procedures of Munson and Walker (1) and Allihn (2), or by any of the several volumetric methods. The amount of glucose multiplied by 0.93 is taken to equal the amount of starch which was in the aliquot. The theoretical factor, 0.9, gives low results.

Occasionally, substances are solubilized by the acid treatment which interfere with a copper reagent or are precipitated by the copper. In these cases, a pretreatment of the acid hydrolyzate is recommended with precipitants such as, phosphotungstic acid. For example, 2 ml. of a 20% phosphotungstic acid solution may be added to the acid hydrolyzate before neutralization and the precipitate which forms filtered. When other substances precipitate with the cuprous oxide in the glucose determination, the amount of cuprous oxide present may be determined by oxidation-reduction methods (3).

Use of this method presupposes the absence of reducing substances in the hydrolyzate other than glucose and that the glucose produced arises only from the hydrolysis of starch. When pentosans are present in the sample which are hydrolyzed to pentoses, a separate determination for pentosans (4) may be made and the results of the starch determination corrected accordingly.

Products wherein the starch is a very minor component and wherein the amount of glucose formed is too small for accurate determination by copper reagents may, after hydrolysis, be analyzed by colorimetric procedures. The author has used 3, 5-dinitrosalicylic acid to determine glucose in the hydrolyzates of starch products from which the acid treatment does not solubilize colored compounds with a high absorption of light in the region of wavelength, 500 mμ. The use of this reagent has been described by Sumner (5).

The reagent is prepared by dissolving 1.00 g. of 3, 5-dinitrosalicylic acid in 60 ml. of 5.00 *N* KOH and adding water to make up to a volume of 200 ml. It is recommended that each operator prepare a standard curve, which is very readily done, to meet his own experimental conditions. One gram of glucose (of known purity and on an anhydrous basis) is dissolved in water and made up to a volume of 1 liter. Several different quantities of the glucose solution are treated with the reagent. The total volume of the test liquid should be 5 ml., for example, 0.5 ml. of the glucose solution plus 4.5 ml. of water. This is introduced into a test tube 6 × 0.75 inches and 10 ml. of the alkaline dinitrosalicylate solution is added with stirring. A blank is also prepared, using 5 ml. of water and 10 ml. of the reagent. The test tubes are immersed in a water bath at 65°C. for 60 minutes. They are cooled and their contents diluted with water to 50 ml. The spectrophotometer is set at 100% light transmission at a wavelength of 500 mμ using the blank and the percent light transmission for the tubes containing glucose is then determined. The standard curve is constructed by plotting mg. of glucose against per cent light transmission.

Aliquots from the neutralized, acid hydrolyzate of the unknown are treated with the reagent in similar fashion. Two or three of these which give per cent light transmissions in range of 30-70 are selected and by locating their values on the standard curve, the corresponding glucose equivalents in mg. may be read. A result in mg. of glucose multiplied by 0.93 is the equivalent amount of starch in the aliquot. The percentage of starch in the original sample may then be calculated. An average is taken of the values determined from the several aliquots.

Several other colorimetric methods have been used for determining very small amounts of glucose and these may be used under suitable conditions. Some of these have been reported rather recently (6).

✓*b. Hydrolysis with Diastase.* In order to overcome some of the problems involved in the determination of starch by acid hydrolysis, as described above, other hydrolytic procedures have been developed which are more specific for starch. .

A weighed quantity of the finely-ground sample, at known moisture content, and representing about 2.5 g. of the dried material is stirred or shaken with 250 ml. of water at room temperature for an hour. The insoluble residue is transferred quantitatively to a small filter, preferably of the Gooch type, of known weight, with the aid of an additional 100 ml. of water. The residue is washed on the filter with 3 portions of alcohol, about 50 ml. each, and then with 5 successive portions of 10 ml. of ether. The residue is dried and weighed and the loss in weight during these extractions noted. Another sample is treated in similar fashion except that it is filtered on a small Buchner funnel. This second residue is transferred to a small beaker with the aid of 100 ml. of water. The beaker is immersed in a boiling water bath and the contents stirred for 15 minutes. The gelatinized product is quickly cooled to 55°C. and 20 ml. of clear malt extract is added. The reaction mixture is maintained at 55°C. for an hour, heated to boiling temperatures for a few minutes and immediately treated a second time with malt extract at 55°C. until the insoluble residue no longer gives a blue coloration with dilute iodine solution. The hydrolysis mixture is diluted to about 250 ml., filtered through a weighed Gooch type filter, and washed on the filter with three successive portions of

50 ml. of warm water. This residue is dried and weighed. The amount of starch in the original sample is calculated as the loss in weight resulting from the hydrolysis procedure, less the loss which was observed during the preliminary extraction procedures.

In this procedure it is assumed that the enzymes in the malt diastase, or other α-amylase containing preparation, liquefies only starch. Malt diastase may be prepared for use in the above procedures by stirring 5 g. of dry malted barley grain, ground to a coarse powder, with 100 ml. of water for an hour at room temperature, followed by filtration. The extract of the ground barley malt should be made just prior to use. Proprietary α-amylase preparations may be used, of course, instead of malt. Those free from proteases and types of enzymes other than amylases may be more advantageously used since less error is involved due to the liquefaction of insoluble material other than starch, which may occur when crude extracts are used.

In order to provide more specificity to the test, one procedure (7) directs a combination of malt diastase liquefaction, very much as described above, followed by acid hydrolysis of the partially hydrolyzed starch liquor and a determination of glucose. The latter portion of this procedure has also been discussed as a method for the determination of starch. However, in the great majority of cases when procedures are used involving diastatic hydrolysis of the starch, the results are too low, compared to results by other methods and it is believed that the major inherent error is due to incomplete liquefaction of the starch in the sample. In grains, for example, all of the starch may not be made available to the enzyme, owing either to the fact that some granules may remain embedded within impervious cell aggregates after grinding or because the starch may be insufficiently dispersed during gelatinization. In products which contain pregelatinized starch, retrogradation may have rendered a part of the starch permanently insoluble in water and inaccessible to the enzyme.

Accordingly, procedures have been developed in recent years which make use of more powerful dispersing agents for the starch and improved techniques for determining the carbohydrate. Several of these are discussed below.

c. *Dispersal in perchloric acid followed by iodine colorimetry.* This method has been described by Nielsen and Gleason (8) and compared with other methods.

If dry, the sample is hydrated by heating with water at 60°C. for 2 hours followed by disintegration in an electric food blender. A known amount of material corresponding to about 2 g. of the sample is treated in the cold with 2 ml. of perchloric acid for from 7 to 10 minutes to solubilize the starch. The perchloric acid is diluted with water to a concentration of 45% before use and cooled. Stirring should be vigorous while the acid is being mixed into the sample. After solubilization of the starch the mixture is diluted to a convenient volume, neutralized with 2 N NaOH and, if necessary, clarified by centrifuging. The solution is then made up to a definite volume and an aliquot is taken to develop a blue color with iodine. The volume of the test solution will depend on the amount of starch in the sample. Not more than 2.5 mg. of starch per 50 ml. of final solution in the colorimeter tubes of 1.25 cm. internal diameter should be used. Stated generally, the fractional transmissions of light should preferably be within the range of 20 to 80%.

Nielsen recommends diluting an 1 ml. aliquot of the original perchloric acid digest with 6 ml. of water and after neutralizing with 2 N NaOH, adding 2.5 ml. of 2 N acetic acid. Then, 0.5 ml. of a 10% KI solution and 5 ml. of a 0.01 N KIO_3 solution are added and the mixture is made up to volume, either to 25 or 50 ml., for measurement of the color value in a Klett-Summerson photoelectric colorimeter. Standard curves are made, treating various starches in amounts between 0 and 2.5 mg., by the above procedure. The color values for each starch are plotted against the weight of starch used. Standard curves vary depending upon the type of starch used, whether it is, for example, potato or pea starch. The amount of starch in the aliquot is determined from its colorimeter value on the appropriate standard curve.

See also, the discussion which follows procedure *e*.

d. Disperal in Calcium Chloride Followed by Measurement of Optical Rotation. Hot calcium chloride solution is an excellent dispersing agent for starch. Its use in determining starch and the factors which influence the precision of the determination have been discussed in some detail by Clendenning and coworkers (9). The method as applied to whole and patent wheat flours or similar products is as follows: The sample is ground to pass a 40 mesh screen and 2.463 g. is made into a smooth suspension with 10 ml. water in a glass beaker with the aid of a stirring rod. Reagent grade calcium chloride s made into an aqueous solution and the density adjusted to 1.20 at 20°C. Then 60 ml. of this solution and 2 ml. of an 0.8% aqueous solution of acetic acid are added with stirring to the suspension of the sample. The mixture s quickly brought to a boil with agitation and boiled briskly for 15 minutes. f foaming persists, a small amount of octyl or cetyl alcohol may be added. Water should be added during boiling to compensate for loss by evaporation. The solution is cooled to room temperature and either 2.5 to 5 ml. of 4% stannic choride or 10 ml. of 5% uranyl acetate is added with stirring. The liquor is transferred to a 100 ml. volumetric flask and brought up to volume with additional calcium chloride solution. The liquor is filtered through 15 m. Whatman No. 12 fluted paper, discarding the first 20 ml. The filtrate is placed in 2 dm. tubes and the optical rotation is measured in the polarimeter using sodium light. The percentage of starch is taken as the reading in degrees multiplied by 10.

The use of uranyl acetate as the protein precipitant is recommended for the determination of starch in corn, barley, rye, rice, grain sorghum, and buckwheat. It is assumed that the specific rotation of starch is +203°, and this appears to be true for a very large number of starches, particularly the cereal starches. Potato starch gives a value more nearly equal to 204° and the legume starches approach 200°.

Earle and Milner (10) have found that the specific rotation of all pure starches in aqueous calcium chloride solution is so close to +203° that this value may be used in all polarimetric analyses using this solvent. They have also found that stannic chloride is a suitable precipitant for protein in corn by a procedure which is outlined as follows. The sample is ground and 2 g., dry basis, are weighed into a 250 ml. beaker. The sample is hydrated with 10 ml. of water and then 70 ml. of an acidified calcium chloride solution is added. The solution is prepared by dissolving calcium chloride in water, adjusting the density to 1.30 at 20°C. and adding glacial acetic acid to adjust the acidity to pH 2.5. The beaker is covered with a watch glass, brought to a boil and held at boiling temperature for 15 minutes with frequent stirring. After cooling, 5 ml. of stannic chloride solution is added. The stannic chloride solution is prepared by dissolving 2.5 g. of $SnCl_4.5H_2O$ in 97.5 g. of the calcium chloride solution. The volume of the starch solution is now adjusted to 100 ml. in a volumetric flask with calcium chloride solution and the liquors are filtered on a folded No. 2 Whatman filter paper. The first 10 ml. are discarded. The solution is polarized in a 10 cm. tube. The percentage of starch is calculated as:

$$\frac{\text{Observed reading} \times 10^4}{203 \times 2}$$

Hoffpauir and Guthrie (11) have observed that stannic chloride appreciably reduces the optical rotation of starch in calcium chloride solution. Therefore comparisons between standards and unknowns should be made on the same basis; in both cases the same protein precipitant should be used. Steiner and Guthrie (12) have developed a polarimetric method wherein uranyl acetate is used as the protein precipitant.

See also, the discussion which follows procedure *e*.

(e) *Dispersal in Calcium Chloride Followed by Iodine-Spectrophotometry.* The author has used hot calcium chloride solutions to dissolve starch products for measurement of light transmission values of the iodine complex for severa reasons. First, because of the ease with which the starch dissolves withou apparent degradation; secondly, because it has been found that the calcium chloride solution allows complex formation between starch, or its amylose, and iodine to proceed normally without interference from fatty acids which are normally associated with many starches (13); thirdly, because of the sensitivity of starch to the iodine reaction and the accuracy with which color intensities may now be determined; and lastly, values may be obtained which, together with polarimetric measurements on the calcium chloride solution, may be used to calculate the composition of the starch as well as the starch content, such as, for example in a series of grain samples.

The essentials of this method are a combination of the procedures for dissolving starch in the product with calcium chloride very much as described by Earle and Milner, and as outlined under *d*, and the iodine-spectrophotometric technique given by Kerr and Trubell (13). After 2 g. of the product are dissolved in 100 ml. of the $CaCl_2$-$SnCl_4$ solution and filtered, a 5 ml. portion is placed in a one liter volumetric flask containing about 700 ml. of water. Then, 20 ml. of an aqueous solution containing 0.5 g. of iodine and 0.75 g. of potassium iodide per 100 ml. are added and the volume adjusted to 1 liter with water. If the original product contained less than 50% of starch, the blue color may be read directly in a spectrophotometer using a cell of proper depth. If the product contained 50% or more of starch, the blue test solution should be diluted immediately. A maximum dilution suggested, on the assumption that the product is all native starch would be fivefold.

Standard curves are prepared for each starch by first dissolving 2 g. of a known starch in 100 ml. of the $CaCl_2$-$SnCl_4$ solution. Then, 5 ml. of this solution together with 20 ml. of the iodine in potassium iodide are diluted with water to 1 liter. The latter is immediately diluted with water so as to obtain, finally, solutions which contain various concentrations of starch from 0.001 to 0.01 g. per 100 ml. The percentage light transmissions of each is measured at a wavelength of 600-620 mμ, using dissolved starch similarly diluted, but without iodine, to set the instrument at 100% light transmission. The logarithm of the per cent light transmission is plotted against the concentration of starch; the logarithm of the per cent light transmission of the unknown test solution is then located on this curve and the concentration of starch to which this point corresponds is noted. Knowing the concentration and the volume of the test solution, one may calculate from the various dilutions made the total amount of starch in the unknown sample.

Obviously, if amylose is used (at lesser concentrations) instead of starch to prepare the calibration curve, the percentage of amylose in the original sample may be determined. From a determination of the optical rotation of the calcium chloride solution of the sample, the amount of starch may be estimated by this alternate procedure as indicated in a previous section and from the two values, amount of starch and amount of amylose one may, if so desired, calculate the composition of the starch in the unknown (14). The writer has applied this combined method to corn entering the mills to test its reliability and has also applied the procedure to a very large number of hybrid corn samples, available only in small quantities in plant breeding experiments, in order to note possible variations in the composition of the starch.

In the above analytical procedure, as well as in several others which have been dicussed, it is presumed that the starch in the product for analysis is substantially in its native state, that is, that it has not been altered chemically either by hydrolysis or derivatization. Also the type, or variety, of starch for which the analysis is being performed should have been ascertained. Starch changes during chemical treatment in its characteristics which are measured in analytical procedures. For example, the specific rotation and iodine color value progressively decrease as starch is hydrolyzed. Also these values are usually less for starch which has been esterified or etherified even though the starch molecules may not have been degraded during derivatization. Furthermore, as has been intimated above, the iodine color value, and to a lesser extent, the specific rotation of starch changes with source or botanical variety. Therefore, in cases where the nature of the starch in the sample is quite unknown, it becomes necessary to separate, preferably by physical means, a representative portion of the starch in the unknown sample and to use this material as the standard of comparison in polarimetric or spectrophotometric measurements. Usually this separation can be made by utilizing the information given in Section II of this text, under the heading, "Preparation."

It may be noted that many of the tests given lead to a calculation of results in terms of pure carbohydrate material. Commercial starch usually contains between 1 to 2% of non-carbohydrate constituents such as, ash, nitrogenous and fatty materials. Therefore, if the purpose of the analysis is to determine the proportion of starch blended into a commercial mixture, the analytical results will give a value which is too low. For example, many commercial starches give a specific rotation on a dry basis of +200° and therefore in practical analysis of commercial mixtures such as baking powders and pudding formulations, the use of a specific rotation value determined from a commercial sample of the starch present would be more suitable.

f. Other Recent Methods and Improvements. Hixon (15) has described a method for the determination of starch in corn samples by taking the difference in weight when 1 g. of ground grain is heated for 45 minutes on a boiling water bath with 100 ml. of an aqueous solution (0.3 g. per 100 ml.) of ammonium persulfate. Correction is made for the water soluble material within the grain, originally. Rask (16) has offered improvements in the A.O.A.C. method wherein starch is dissolved in hydrochloric acid and precipitated by the addition of alcohol. Watson and Whistler (17) have described a rapid and improved technique for the determination of starch by iodine color tests. Pucher and coworkers (18) have determined starch in plant tissues by extraction with perchloric acid, precipitation of the starch with iodine, hydrolysis of the precipitate with acid and a determination of glucose. Weinmann (19) prefers to use taka-diastase to pretreat plant material whereas van Voorst (20) uses pancreatic amylase after which, in both cases, hydrolysis is completed with acid and glucose is determined.

✓**2. Analysis of Starches.** Substances most usually associated with starches are moisture, inorganic elements or compounds, nitrogenous bodies such as protein or their split-products and lipids. In addition, some starches may contain small amounts of plant fiber, but with modern milling practices fiber as such should not be present in more than trace amounts. On the other hand some varieties of refined starch may have pentosans so closely associated with the starch that they are not removed in the milling process. Thus, Baker *et al.* (21) found 0.4% of pentosans in prime quality wheat starch and as high as 14% in some secondary samples when analyzed by the method of Schmidt-Nielsen and Hammer (22). Other methods for determining pentosans have been referred to in Section I. Usually, however, pentosans may be neglected since blank determinations, using pure glucose, have been found to give a "pentosan" value by some methods, of the order of that obtained from prime starch (9).

Naturally, some starch products may contain adjuncts deliberately added for some specific purpose. For example, borax, or urea are oftimes added to adhesive formulations to improve the tack or drying qualities of the starch product. All of these additions cannot be anticipated and when qualitative tests indicate their presence, they may be determined by the methods commonly employed in quantitative analysis.

Obviously, since optical rotation measurements may be used to determine starch, the "purity" of starch may be most conveniently determined by polarimetric measurements when the purity value is all that is required. When the impurities are to be determined according to their type, or class, the procedures given below may be employed.

✓*a. Moisture.* The determination of moisture in starch and starch products has received a very large amount of study. The principal problem involved in common methods employing heat is to expel the last traces of moisture from a material in which it is held by tremendous physical forces without inducing a chemical degradation of a relatively labile organic material. Thus, the author has not succeeded in reducing the weight of some starch samples by placing them in a closed chamber over phosphorus pentoxide to a value which could not be further reduced by subsequent heating the starch in a vacuum oven at 120°C. Very much less difficulty is experienced in drying starch derivatives, such as amylose triacetate, over phosphorus pentoxide. From these facts it may be concluded that the water in a starch sample is of two or more types, free moisture, such as that which may be loosely absorbed and adsorbed on the surface of the starch particles, and water which is held by association through secondary bonds to the hydroxyl groups of the starch molecules. The latter is held by forces which no doubt vary in strength depending on the hydroxyl group of the starch to which the water molecule is held, whether it is a primary alcohol group which forms a strong hydrogen bond, or a secondary alcohol group which ordinarily forms a weaker bond. Furthermore, it has been known for many years (23) that when starch has been extensively dried to very low residual water content changes are induced in the starch. This dried material will not, over limited periods of time, take up the same amount of water vapor that undried starch will, when both are exposed to the same relative humidity, or water vapor pressure. Consequently, for the present in analytical work one must neglect the academic question as to what really constitutes moisture in starch and define this value in terms of procedures used to obtain it. Two commonly accepted procedures are as follows.

A starch sample is weighed in a receptacle with a tight fitting cover and of such shape that the sample used does not cover the bottom to a depth of more than about 2 mm. The cover is removed and the receptacle placed within a vacuum oven at 105°C. The sample is heated under a pressure of about 25 mm. until substantially constant weight is obtained. For the ordinary types of granular starch in powdered or fine pearl form, 4 hours will suffice. Pressure within the oven is then equalized by admitting air which has been passed over or through a highly efficient drying agent. The cover is placed on the receptacle which is removed from the oven and cooled in a desiccator over P_2O_5 or other drying agent. The loss in weight during the heating period is taken as being equal to the moisture.

Technique commonly employed in quantitative measurements is followed in this and the other procedures given. The receptacle is preheated in the oven and cooled in a desiccator before use. For moisture contents of 10% and less, sample weights are determined on an analytical balance to five significant figures.

A second method which is used, particularly for starch products of high moisture content, or those which do not dry by heat very readily, is distillation of the moisture with an immiscible liquid which boils at a temperature above that of water, such as, toluene or xylene. Special equipment with ground glass joints is now made for this determination and this is preferable to an assembly made from the usual laboratory glassware with connections made with corks or stoppers.

Sufficient sample is weighed to give about 5 ml. of condensed moisture. This is placed in an Erlenmeyer shaped distilling flask with about 100 ml. of toluene. Dry sand or glass beads may be added to prevent bumping. The flask is connected by a tube to a special chamber at the top of which a water-cooled condenser is attached. A narrow tube, graduated in tenths of a milliliter, is connected to the bottom of the chamber which is also filled with toluene at the start of the determination. The contents of the distilling flask is now brought to a boil and distilled slowly so that about 2 drops per second fall from the condenser into the receiving chamber. Water will separate and sink to the bottom of the graduated tube; the toluene overflows back into the distilling flask. When substantially all the water has been distilled, the heating may be increased so that the distillation rate is raised to 5 drops per second. Droplets of water in the condenser and chamber may be washed down by carefully pouring toluene into the top of the assembly. Droplets of toluene trapped with the condensed water in the graduated tube may be liberated by introducing a fine, clean wire probe. The tube is cooled and the volume of water read. This value is then calculated as percentage moisture based on original weight of sample.

Sair and Fetzer (24) have made a study of this method and have compared it with other procedures for the determination of moisture. More recently, Seeborg (25) has compared several methods for the determination of moisture, as well as protein and ash in cereal products. Two other methods have come into use within recent years. The first involves extraction of water in the sample with absolute methanol and a titration of the water in this solution with the now generally known Karl Fischer reagent (26, 27). The second involves measurement of electrical properties of a starch product such as its specific inductive capacity (28), conductivity or dielectric constant (29) or some other property which changes with moisture content.

The application of a titration with Karl Fischer reagent to starch products has received considerable study. Schroeder and Nair (30) have recently reviewed the subject and have compared results obtained by titrating corn starch with other methods. It was found that the results of titration were intermediate to those obtained by toluene distillation and by oven drying to constant weight.

Several instruments are now available to determine moisture by electrical measurements and these are finding favor, particularly in routine analyses of grain or cereal products. Groves and King (28) have presented an interesting discussion of the principles involved in the construction of a laboratory instrument for measuring changes in specific inductive capacity and have outlined the limitations of the method which appear to be general for other electrical methods. These are primarily, that the electrical measurements require calibration against moisture contents determined by other methods such as oven drying and that a calibration determined for one type of product does not necessarily apply to another. Furthermore, even for samples of the same type, the degree or manner of packing or compressing the sample in the electrical chamber may appreciably affect the result.

b. Ash. Ash is usually taken as the incombustible residue which remains after placing a weighed sample in an appropriate dish into a furnace heated to 600°C.

When qualitative tests indicate the presence in the sample of inorganic constituents which are volatile at 600°C., these should be determined independently by appropriate quantitative procedures.

c. Nitrogen. Nitrogenous bodies are usually determined by the Kjeldahl method or some modification of this procedure. A weighed sample, about 3-5 g. for most starch products, is digested in a Kjeldahl flask by boiling with 25 ml. of concentrated sulfuric acid, and using about 0.2 g. of copper sulfate as catalyst. Boiling is continued for 30 minutes after the reaction mixture becomes clear. The flask is cooled, diluted with 200 ml. of water and a few pieces of granulated zinc and 25 ml. of 4% sodium sulfide solution are added. About 50 ml. of 45% sodium hydroxide solution is cautiously run down the side of the flask which is then connected to a condenser. The outlet of the condenser should extend into a measured quantity of standard acid solution. The flask is gently shaken to mix the contents and heat is applied to distil the ammonia from the reaction flask into the standard acid. The first 150 ml. of distillate usually contains all of the ammonia. The excess acid in the receiver is titrated with standard sodium hydroxide solution, using methyl red as an indicator.

After a determination of the amount of ammonia distilled and calculation of the results as nitrogen, it is common practice to report values as per cent protein by multiplying the percentage of nitrogen by a factor. A factor of 6.25 is ordinarily used except for wheat starch products in which case, 5.7 is preferred.

Starches usually contain such a small amount of protein that extensive precautions should be taken in making the determination. The reagents, particularly the sulfuric acid, sodium hydroxide, and copper sulfate should be free from nitrates or ammonium salts. The distillation unit should be swept clean before use by distilling about 200 ml. of water through it. The water used should be free of ammonia and the flask used to collect the distillate should be protected from possible alkaline fumes in the laboratory. Still heads should be installed before the condenser to prevent entrainment of sodium hydroxide in the distillate. Even with these precautions, blank determinations should be made using a substance known to be free of nitrogen and a correction made on test results by subtracting the actual value (not as percentage) obtained with the blank.

Starch products which are found to contain such added adjuncts as urea or ammonium salts should be analyzed by appropriate methods for these substances, and naturally the results by the Kjeldahl test should be expressed as nitrogen, not protein.

✓ *d. Lipids.* The lipids in starch are usually of three types, the fatty acid glycerides of natural oils or fats which occur in grain or plant tissue and which may have been adsorbed by the starch, phospholipids, and fatty acids which may be associated in a molecular complex with starch molecules. When a distribution between the three classes is not required, total fat may be determined by hydrolyzing the starch in an aqueous acid solution and extracting the fats in the hydrolyzate by use of a suitable solvent. This procedure is referred to as, "fat by hydrolysis."

Two-gram samples are weighed out in duplicate in Erlenmeyer flasks. To each is added 25 ml. of 2 *N* HCl. The flasks are shaken to form a slurry, loosely covered and immersed in a boiling water bath. Heating is continued until samples from one flask give no coloration with iodine solution. The other flask is heated an additional 15 minutes, then removed and cooled. The contents are transferred to an extraction tube provided with a draw in its upper portion. The hydrolysis flask is rinsed with a mixture of 10 ml. of ethanol, 30 ml. of ethyl ether and 30 ml. of petroleum ether, and this is poured into the extraction tube. This is shaken and allowed to stand until the upper layer is clear. The upper layer is then drawn off and filtered into a dry, weighed dish. The solvent is evaporated slowly on a steam bath. The residual liquor in the extraction tube is treated two additional times with a mixture of 30 ml. of ethyl ether and 30 ml. of petroleum ether as before and passed through the same filter into the evaporating dish. When evaporation is complete, the dish is placed in an oven at 100°C. for 1 hour, then cooled and weighed. A blank determination is run on the reagents and solvents used and any amount found is deducted from the weight of lipids found for the test material.

Extraneous fat in starch products may be determined by the usual Soxhlet extraction technique using redistilled carbon tetrachloride and exercising the usual precautions.

✓ **3. Qualitative and Quantitative Determination of Starch Modifications.** Determination of the extent and nature of the modification of starch from its natural state presents several problems some of which apply to high polymers in general and others to starch in particular. For example, the modification of starch may be followed by viscosity measurements and in the simple case where the starch molecules are split by a mild acid hydrolysis, as in the production of many useful textile and paper sizes, the viscosity will decrease as a function of the extent to which the hydrolysis has proceeded. This will be found to be true whether aqueous pastes are employed for consistency measurements, as outlined in a previous chapter, or whether the starch is dissolved in a suitable solvent, such as sodium hydroxide solution, and actual viscosity measurements performed. Indeed, viscosity measurements are probably the most sensitive test by which the degradation of high polymers may be determined. On the other hand failure to observe a reduction in paste viscosity does not necessarily indicate that the starch is unmodified. Many treatments increase the paste consistency and one of these may have been used either alone or in combination with a degradative reaction.

Again, starch is substantially insoluble in cold water. The lack of solubility is not because the molecules are too large to dissolve nor because of an insufficient number of hydrophilic groups on the chemical structure of the molecules, but rather it is due primarily to physical forces which hold these molecules together in an organized state, the granule. Although it is true that a hydrolytic treatment of starch, both in the wet and dry states, does tend to develop cold water solubility and a determination of the percentage of cold water-soluble solids is a very common analytical test to measure modifications such as dextrinization, the result is significant only in comparing with each other, products made by the same type of treatment; the result on an unknown may mean very little, in itself, unless the nature of the soluble solids is also determined. The reason is that the physical structure of the granules which is responsible for insolubility in native starch could have been disrupted, in whole or in part, by physical treatment.

Again, in the case of starch, one component called amylose possesses a rather specific property of forming a colored complex with iodine. It is well known that as starch is hydrolyzed by acids or enzymes and the chain length of the amylose molecules become smaller, the characteristic features of complex formation between amylose and iodine change (31, 32). However, the use of this test on starch is complicated by the fact that even a very limited chemical treatment of starch, such as certain types of etherification, very materially decreases the ability of the amylose component to form a characteristic complex with iodine.

Therefore, it is often necessary to apply a variety of tests to starch and from a combination of the results deduce the probable nature and extent of modification. In cases where the deduction does not appear to be conclusive, it may be confirmed by some specific test.

The use of specific chemical tests on starch also involves problems, some of which are not ordinarily encountered in the field of low polymer chemistry. The chief one of these arises from the fact that starch may be profoundly modified in its properties with very little chemical change and therefore a very large variety of useful products may be made with so little treatment that it is difficult to determine by orthodox procedures. For example, starch may be derivatized to form a glycolate (33) and there are three hydroxyl groups on each glucose monomer in the starch molecule which may react. However, the introduction of as little as one ether group in every 50 glucose units in corn starch changes the colloidal properties so that the derivative resembles a waxy starch and the addition of a few more per hundred converts the starch into a cold water-soluble, highly viscous material, resembling the natural gums. On the other hand, etherification or derivatization with bifunctional reagents such as epichlorohydrin and the establishment of probably less than one cross-link per 100 glucose units converts starch into an inert powder which is not dispersible even in 5 *N* NaOH or boiling water. Thus, commercial derivatives of starch may possess only a very small percentage of substituent groups, a value hardly larger than the experimental error of available chemical methods for the determination of these groups. Consequently, unless the starch product is obviously derivatized to a relatively high level, it would seem profitable to examine the physicochemical properties, at first at least, in order to ascertain the probable type and degree of the modification. The more commonly used tests which have been developed in this branch of starch chemistry are given in the following sections. Following these is a summary table and several illustrative examples, indicating how the use of these tests may be combined into a qualitative procedure, at least, to determine the nature of an unknown starch product.

✓*a. Solvent Solubility.* The most important test in this respect is solubility in water.

Two grams of the powdered or ground material is made into a uniform slurry with about 50 ml. of water and then diluted to about 180 ml. in a 200 ml. volumetric flask. This is shaken for an hour at 25°C. and made up to volume. The solution is then centrifuged or filtered. When filtration is employed, the first 25 to 50 ml. of filtrate are discarded. Exactly 50 ml. of filtrate are evaporated to dryness in a dish on a steam bath, followed by drying to constant weight in a vacuum oven at 110°C. Two hours usually suffices except for highly soluble starch products. The weight of evaporated solution in grams divided by 0.005 (or multiplied by 200) is the percentage of cold water-soluble solids in the original sample.

All native starches are substantially insoluble in water. The acid hydrolyzed and oxidized, thin boiling starches of commerce develop some water soluble solids during manufacture but these are usually washed from the starch during the final phase of the process. Extensive degradative reactions, hydrolysis, oxidation and dextrinization, lead eventually to cold water soluble products.

The granule structure is weakened also during derivatization and solubility develops depending on the nature and extent of the derivatization. For example, the methyl, ethyl and propyl ethers and hydroxy ethers are ordinarily insoluble in water when the degree of substitution (D.S.) is of the order of 0.1 (one substituent group for every ten glucose monomer units). However, as the D.S. approaches the range of 0.5 to 1.0 many of these ethers become completely soluble in cold water. In the higher ranges of D.S., 2 to 3, water-insolubility again develops but the product may then tend to become soluble in organic solvents such as, acetone, chloroform and pyridine. Even in this range of D.S., many starch derivatives may show limited solubility, or even insolubility in organic solvents owing to residual granule structure, or physical association between molecules unless the starch is degraded, intentionally or otherwise, into a low molecular weight range.

Physical treatment of starch may also be used to develop water solubility. The most common of these is a gelatinization treatment during manufacture followed by roll-drying or spray-drying the paste. Obviously, when the process used to develop water solubility is substantially physical in nature the water-soluble portion will exhibit properties of native starch, high iodine color value, relatively low reducing value and high viscosity.

As indicated above, solubility in organic solvents in the cold except the strongly basic ones such as ethylenediamine, is indicative of a high degree of derivatization. Some data on this subject has been given in Chapter X. Complete insolubility in all solvents, both organic and aqueous alkali, or other basic solutions, may indicate that the starch molecules have been cross-linked by bifunctional reagents.

b. Viscosity. Paste viscosity or consistency measurements, particularly when made in continuous pasting and recording instruments are very useful in characterizing starch products. These have been outlined in Chapter VI.

Starches are soluble at room temperature in strong bases and several of these solvent systems have been used to determine the solution viscosity of starch and starch products. When microscopic examination shows that the product is comprised of intact granules a preliminary heating or gelatinization is recommended, however, in order to increase the rate of solution in the solvent. A convenient method is gelatinization in hot water followed by solution in cold 1 *N* KOH, a procedure developed by Lansky *et al.* (34), which is as follows:

Two and one half grams of sample, dry basis, are stirred into about 300 ml. of water. After the material is dispersed, it is heated for 30 minutes in a boiling water bath, then allowed to cool to room temperature, stirring being continued throughout the heating and cooling periods with an agitator extending through a perforated watch glass cover. Then, 100 ml. of 5 *N* KOH solution is added and the solution is made up to a volume of 500 ml. with water at 35°C. This 0.5% starch solution in 1 *N* KOH is diluted with 1 *N* KOH to give samples of various concentrations such as, 0.40, 0.30, 0.20 and 0.10%. Viscosity is determined at each concentration with a Cannon-Fenske No. 100 pipette, maintained in a thermostatically controlled bath at 35.0 ± 0.2°C. Flow time is determined with a precision of ±0.05 second (average deviation from the mean).

Both the 1 *N* and 5 *N* KOH solutions are accurately made using titration with potassium acid phthalate for standardization. The flow time for the 1 *N* KOH solution is also determined in the viscometer at 35°C. The specific viscosity, η_{sp} for each concentration is calculated as follows:

$$\eta_{sp} = \frac{\text{flow time of solution} - \text{flow time of } 1\ N\ \text{KOH}}{\text{flow time of } 1\ N\ \text{KOH}}$$

The specific viscosities, divided by concentration of starch product (in grams per 100 ml.) are determined and these values (η_{sp}/C) are plotted against concentration. The curve is extrapolated to zero concentration in order to calculate the intrinsic viscosity, $[\eta]$.

The intrinsic viscosity of starch (and its fractions) decreases as it is hydrolyzed, in a manner indicated in the table given in Section *f*. The intrinsic viscosity also decreases with oxidation and dextrinization; values for the majority of corn pyrodextrins are below 0.25. The intrinsic viscosity of many corn starch derivatives of low degree of substitution is not materially different from the underivatized starch. For example, when an acid-modified, 20 fluidity starch is etherified to a D.S. of 0.1, it will be found that its intrinsic viscosity is not materially different from that of the underivatized, 20 fluidity starch which is 0.9. The paste consistencies of the two starches, however, may be quite different. It follows from the above that intrinsic viscosity may be used to determine the extent of degradation of the carbohydrate molecules in a derivative, at least of the water dispersible type.

Esters may be saponified by basic solvents, but viscosity values such as those in hydrazine hydrate (35) may still be significant. The viscosity of highly derivatized starch products may be determined in appropriate solvents such as the methyl ether in chloroform (36), the nitric acid ester in acetone (36) or ethyl acetate (37), and the acetic acid esters in chloroform (38) or methyl acetate (39).

c. Iodine Reaction. A very important characteristic of starch is the manner in which it reacts with iodine to form a complex. There are several observations which are of importance in this connection. In following the reaction by potentiometric titration, the amount of iodine bound by the starch may be calculated as well as the characteristic potential at which the starch reacts. The procedure, originated by Bates *et al.* (40) has been outlined in Chapter ~~XVIII~~. XVII. [illegible].

The procedure for the determination of the percentage weight of iodine bound by a starch sample, often referred to as iodine affinity, has frequently been modified. A recent one, recommended by Lansky *et al.* is as follows (34):

A bright platinum electrode is used in conjunction with a low resistance calomel electrode (Leeds and Northrop No. 1199-11, or Coleman No. 3-070 with saturated KCl junction). Potentials may be determined with an ordinary pH electrometer, adaptable to millivolt readings, although use of a Leeds and Northrop Type K bridge and No. 2420-C galvanometer provides much simpler and more precise operation. To calibrate the electrode system, 373 mg. of potassium chloride and 830 mg. of potassium iodide are dissolved in 100 ml. of water and titrated with standard iodine solution between E.M.F. limits of 230-280 millivolts. The solution should be stirred mechanically during titrations. The standard iodine solution is 0.05 *N* with respect to KI, 0.05 *N* to KCl, and contains exactly 0.200 mg. of iodine per ml. This standard is most conveniently prepared, just prior to use, by tenfold dilution of a stock iodine solution, which is standardized against arsenious oxide. There is no change in salt concentration during titration since the solution and the iodine standard are both 0.05 *N* with respect to potassium chloride and potassium iodide. The results of the titration are plotted and from this curve a calibration chart is prepared giving the total number of milligrams of free iodine in solution for each half millivolt reading over the range 230-280 mv.

Starch products should be purified before the determination in order to remove impurities or adjuncts which affect the iodine affinity value. Usually, a Soxhlet extraction for 24 hours with ethyl alcohol will suffice. The sample is then dried and ground to pass a 40 mesh screen. Granular, unmodified starches may be prepared for analysis by gelatinization in boiling water at 1% concentration, disintegration in a Waring Blendor for 5 minutes, and flocculation of the starch substance by the addition of excess methanol. Modified, or so-called thin-boiling starches, do not require pregelatinization. An appropriate sample (e.g., 100 mg. of normal starches or 200 mg. of waxy starches) is transferred to a dry 250 ml. beaker, to which 5 ml. of 1 *N* KOH solution is added by pipette. The sample is dispersed in the alkali by trituration with a stirring rod, care being taken to avoid formation of difficultly soluble lumps. The mixture is allowed to stand with occasional stirring for 1 hour, or until a perfectly clear solution is obtained. The solution is then neutralized to methyl orange with 0.5 *N* HCl, 10 ml. of 0.5 *N* KI are added and the solution is diluted with water to 100 ml.

The starch solution is now titrated potentiometrically. After each addition of iodine, the solution should be allowed to equilibrate for two minutes before determining the E.M.F. For each point of the titration, the free iodine in solution is calculated from the corresponding E.M.F. of the calibration chart, and this amount is deducted from the total amount of iodine added at that point, in order to estimate the amount of iodine bound by the starch. Bound iodine is plotted against free iodine for each point in the titration. The end of complex formation between the starch product and iodine is usually observed by a rapid change in slope of the curve. The upper, and more horizontal portion of this curve is extrapolated to the zero axis and the amount of bound iodine found at this point, expressed as percentage weight of the starch sample, dry basis, and is taken as the iodine affinity of the starch.

During the early stages of acid hydrolysis, such as in the manufacture of thin boiling starches, the iodine affinity changes very little, compared to reduction in viscosity, although the characteristic potential at which linear starch polymers react decreases with chain length (31, 41). During oxidation, iodine affinity decreases more noticeably with a decrease in viscosity and in dextrinization, the decrease in iodine affinity is very pronounced (42). An outstanding characteristic of the water dispersible, low D.S. derivatized starches is their low iodine affinity, as will be noted from the table in Section *f*.

Iodine affinity is also a function of composition of a starch and may be used to determine the A-fraction, or "linear polymer" content of a starch. The amyloses usually have an iodine affinity of approximately 19. Therefore, if a starch, such as defatted corn starch, has an iodine affinity of 5.4, the amylose content of the starch may be calculated as $5.4/19 \times 100$, which is 28%. Waxy starches, being substantially all branched polymers, have an extremely low iodine affinity for a given viscosity.

Composition may also be determined by spectrophotometric methods to follow the iodine reaction (13, 43, 44). The method of Kerr and Trubell (13) for the determination of the linear polymer content of starches is as follows:

Pure linear fractions from each type of starch are prepared by the method of Kerr and Severson (45). Branched standards may be prepared, which give no blue color with iodine by alcoholic subfractionation of B-fractions (46). For each starch, various ratios of linear and branched fractions are taken to make 0.100 g. samples. These are stirred into 3.33 ml. of 2 *N* KOH. When dispersion is complete, the volume is made up to 10 ml. with stirring to form a clear solution. This is then transferred to a two liter volumetric flask with about 1.5 liters of water and the pH is adjusted to 5.0 with HCl. Then, 20 ml. of a solution which contains 0.5 g. of iodine and 0.75 g. of potassium iodide per 100 ml., are added and the volume is made up to 2 liters with water. Exactly 20 ml. of this solution is diluted with water to a volume of 50 ml. and the per cent light transmission is determined at a wavelength of 610 mμ in a 13 mm. cell of a Coleman Spectrophotometer. Composition is plotted against the logarithm of the per cent light transmission according to the manner indicated in Fig. 106 for corn starch.

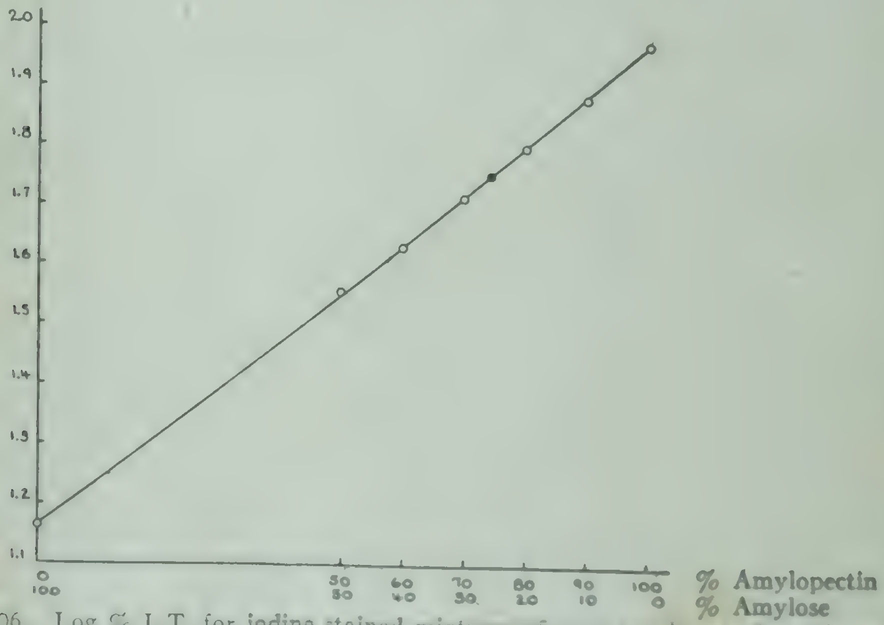


FIG. 106. Log % L.T. for iodine stained mixtures of corn amylose and amylopectin

Composition of an unknown is determined by dissolving, staining and diluting 0.100 g. of the purified sample in the manner indicated above and by noting the percentage values on the standard curve to which the logarithm of its per cent light transmission corresponds.

The wavelength of minimum light transmission for the iodine complex varies with the average chain length of the linear polymer present and this relationship may, accordingly, be used to estimate the extent of hydrolytic scission. Chain lengths between D.P. 500 and 1000 show a minimum at a wavelength of 645-655 mμ, those in the region of D.P. 200-300, about 610-620 mμ, chain lengths of D.P. 100, about 600 mμ, and very short lengths, as low as 550 mμ.

d. Alkali Number. The alkali lability of a starch product may conveniently be determined as the alkali number according to the method of Schoch and Jensen (47).

Five hundred milligrams of a powdered starch sample are introduced into an 8-oz. Pyrex nursing bottle to which 10 ml. of water are added. The bottle is gently swirled to wet and suspend the sample and 25 ml. of 0.4 N NaOH is added. After the starch is uniformly gelatinized, 65 ml. of hot water is added and the bottle is capped with a gum rubber cap, marketed for this type of bottle, pierced with a needle hole to provide an exit for steam. The bottle is placed in a vigorously boiling water bath and heated for exactly 60 minutes. The bottle is then taken out, placed in cold water, the cap removed and about 75 ml. of cold water is added. The excess alkali in the starch solution is determined by titration with standard acid to pH 8. Thymol blue may be used as an indicator. The standard acid used may be 0.2 N H_2SO_4 and a blank should be run using 25 ml. of the 0.4 N NaOH. The alkali number is then calculated as follows:

$$\frac{(\text{ml. of acid to titrate blank-ml. of acid to titrate sample}) \times \text{normality of acid} \times 10}{\text{weight of sample on dry basis}}$$

A correction should be made when testing alkaline or acid starch products by performing a separate titration on the original product. A recommended procedure is to gelatinize 1 g. of the starch in 50 ml. of hot water and to titrate the paste with either standard acid or alkali, using thymol blue as an indicator.

A slightly different technique is suggested for starch products which gelatinize in cold water. The sample is introduced into a perfectly dry bottle and wetted with 1-2 ml. of benzene. Then 25 ml. of 0.4 N NaOH are added with agitation, followed by 75 ml. of hot water. This procedure permits rapid and complete dispersion of the sample which otherwise might form insoluble lumps.

The alkali number may be used to estimate the extent of hydrolysis in a starch product since the value rises progressively as the reaction proceeds. During oxidation, however, the value slowly declines and therefore this test may be used to distinguish the two types of degradation, as measured by viscosity. Dextrins behave still differently, particularly the British gums.

During the early part of dextrinization, the alkali number increases, as in hydrolysis but during the latter stages, the value decreases. The alkali number may decrease materially during derivatization.

In the range of D.P. 200-800, at least, the alkali number of branched starch molecules is materially less than of linear molecules of the same molecular weight.

Native starches have different alkali numbers such as those for corn, potato and tapioca shown in the table in Section *f*.

e. Reducing Value. The reducing value for a starch product may be determined by use of any one of several reagents, such as, alkaline copper solutions (48), ammoniacal silver (49), hypoiodite (50), dinitrosalicylate (51) and alkaline ferricyanide. Although all of these procedures suffer from one or more defects to various degrees, such as, incomplete dispersal of the starch, oxidation of the starch molecules at places other than the original terminal reducing group, and other degradative side reactions, the values obtained by rigid adherence to a specified technique are of value oftimes in cataloging the type of starch product. The alkaline ferricyanide oxidation is, perhaps, the simplest to perform and the values obtained are reproducible to a satisfactory degree.

The reagents required are:

(a) 16.5 g. of potassium ferricyanide and 22 g. of anhydrous sodium carbonate made up with water to 1 liter.

(b) 200 ml. of glacial acetic acid, 70 g. of potassium chloride and 20 g. of zinc sulfate, heptahydrate made up with water to 1 liter.

(c) 20 g. of potassium iodide made up with water to 100 ml.

(d) 0.05 *N* standard thiosulfate solution.

Two hundred and fifty milligrams of starch sample is agitated in an Erlenmeyer flask with 25 ml. of water. When the sample has been suspended, it is heated in a boiling water bath for 2-5 minutes further to disperse the starch and then cooled. Exactly 25 ml. of the alkaline ferricyanide solution is added with agitation and the mixture is heated exactly 15 minutes in a boiling water bath. The flask should be covered with a small inverted beaker or watch glass. The flask is removed from the bath and quickly cooled under a stream of running cold water. Then 60 ml. of the zinc sulfate-acetic acid reagent is added and after thorough shaking, 20 ml. of the 20% potassium iodide is added. The liberated iodine is titrated with 0.05 *N* thiosulfate. The starch sample usually acts as its own indicator with the iodine.

Blanks are run, as above, with 25 ml. of water and no starch sample

Ferricyanide number is calculated as the milliliters of tenth normal thiosulfate per gram of starch, dry basis, as follows:

$$\frac{(\text{ml. } Na_2S_2O_3 \text{ for blank} - \text{ml. } Na_2S_2O_3 \text{ for sample}) \times \text{normality of } Na_2S_2O_3}{\text{weight of sample, dry basis} \times 0.1}$$

The value for native starch is relatively low, being about 1.00 for corn starch.* This value increases progressively as starch is hydrolyzed. The value for maltose is 270 and for dextrose, 310. Accordingly, the method may be used to follow the hydrolysis of starch, particularly by enzymes, such as has been done by Gore and Steele (52) and by Kerr and coworkers (13, 45, 46, 53-55).

For highly hydrolyzed starch samples, less than 0.250 g. should be used and when liquid samples are employed, as from hydrolyzates, small aliquots may be taken and diluted with water to make a 25 ml. sample. If the ferricyanide is exhausted during the test due to the use of too large a sample, no blue color will develop when the potassium iodide is added.

f. Qualitative Procedure for Starch Products. Table XXXVII shows representative values by selected tests described above for several starch samples tested in the laboratory of the writer. These are given primarily to illustrate a method which may be employed to determine the nature of unknown starch products. Three of these are listed (samples A, B and C), together with their analytical values at the bottom of Table XXXVII.

The first two of these unknowns were found to consist, by microscopic inspection, of corn starch granules, or granules closely resembling corn starch, and were substantially insoluble in cold water. Unknown A had an intrinsic viscosity of 0.60 in 1 N KOH at 35°C., which characterizes it as an acid-converted, 60 fluidity corn starch. However, the iodine affinity of 3.3 is subnormal for a starch of this grade and this suggests that the starch was also partially derivatized. The low alkali number confirmed this view. Moreover, since this alkali number was depressed to about only one third of the normal value for a 60 fluidity corn starch, it was concluded that of the common etherifying reactions, formation of a low D.S. glycolate was the indicated manufacturing procedure which had been superimposed on acid hydrolysis. A test for carboxymethyl groups, by procedures given in the next section, gave a result indicative of a degree of substitution of 0.10 per glucose residue, thus confirming the conclusion that the unknown is an acid-modified, 60 fluidity corn starch glycolate of 0.10 D.S.

Unknown B had an intrinsic viscosity normal for unmodified corn starch. The very low iodine affinity of the starch suggests etherification to an inter-

---

* Ferricyanide number for corn A-fraction, 1.45, B-fraction, 0.45.

TABLE XXXVII

Properties of Starches and Starch Products

Starch	Intrinsic viscosity 1 *N* KOH at 35°C.	Iodine affinity	Alkali number
Tapioca starch	2.05	3.30	3-5
A-fraction	3.45	18.50	8.5
B-fraction	1.25	0.00	4.5
Potato starch	2.00	4.15	6.0
A-fraction	1.75	19.05	14.0
B-fraction	1.45	0.17	3.6
Corn starch	1.55	5.30	9.0
A-fraction	1.15	19.35	22.0
B-fraction	1.25	0.45	4.5
Corn, 20 fluidity starch	1.05	4.90	11.5
40 fluidity starch	0.90	4.82	13.0
60 fluidity starch	0.60	3.75	16.5
75 fluidity starch	0.50	3.30	21.0
90 fluidity starch	0.35		26.5
Corn starch glycolate			
0.05 D.S.	1.50	4.10	3.0
0.45 D.S.	1.45	0.80	0.8
Corn starch, etherified			
0.10 D.S.	1.55	3.50	4.8
0.25 D.S.	1.55	3.25	3.7
0.50 D.S.	1.50	2.75	3.2
Corn starch, 75 fluidity etherified			
0.25 D.S.	0.50	2.85	7.8
Waxy maize starch	1.50	0.26	7.5
Unknown sample A	0.60	3.30	5.1
Unknown sample B	1.50	0.54	3.1
Unknown sample C	1.95	4.02	6.2

mediate degree, sufficient to make it a cold water-soluble product. However, the insolubility of the product eliminated this possibility. Although the alkali number was subnormal for corn starch, the value indicated only a very small amount of derivatization, if any. Thus, the low iodine affinity for the product must also have been characteristic of the parent starch which is, accordingly, a waxy maize. On heating the starch in water it pasted at a retarded rate compared to normal waxy maize or to the common low D.S. ethers of this starch. Moreover, the paste assumed a very short consistency even after prolonged cooking, suggesting that dispersion of the granules was being prevented because of a cross-linking reaction between constituent molecules. This conclusion was confirmed by treating alkali-waxy maize starch with a small amount of epichlorohydrin and producing a starch which had analytical and paste characteristics identical to the unknown.

Unknown C was completely soluble in cold water, forming a clear viscous dispersion which resembled the natural gums. However, the intrinsic viscosity was too high for an etherified corn starch of medium D.S., which it approached in colloidal properties. Under the microscope, granule structure was not in evidence in the dry white powder, suggesting that water-solubility had been accomplished by a gelatinization procedure during manufacture followed by drying. The intrinsic viscosity was of the order for either potato or tapioca starch. The iodine affinity was almost normal for an underivatized, and otherwise unmodified potato starch. The alkali number of 6.2 confirmed this conclusion. The product is, accordinglly, a roll-dried, but otherwise unmodified potato starch.

The number of tests indicated in Table XXXVII, could of course, be extended and many of these are also of value in the examination of the large number of starch products which are found on the market. For example, the ferricyanide number is of value in distinguishing between products highly modified by acid and pyrodextrins, or in differentiating between pregelatinized products converted by several methods such as, acid hydrolysis, enzymolysis or oxidation. No attempt has been made at this time to make the list of tests or the variety of starch products complete in the table. Furthermore, the precise values obtained by use of the tests described often depend on apparently minor variations in technique among operators and it is therefore believed preferable for each operator to prepare a table of analytical results from known samples by his own technique, unless a broad qualitative classification of unknowns is all that is desired. As indicated earlier, the above examples are given primarily to illustrate the use that may be made of analytical methods which have appeared in recent starch literature, and to show how these may eventually be combined into a systematic method of qualitative analysis.

g. Quantitative Tests for Groups Attached to, or Created in Starch Molecules. Of the groups chemically added to or formed in the starch molecules the following are the most frequently encountered in starch products: ester, ether, carboxyl, and carbonyl groups.

(1) Ester Groups. Simple ester groups, such as acetyl and propionyl, once they have been qualitatively identified, are readily determined by saponification and titration of the liberated acid. A method for starch acetates, adapted from procedures given by Murray *et al.* (56) is as follows: 0.500

g. of sample is mixed with 20 ml. of pyridine in a loosely stoppered Erlenmeyer flask. The mixture is heated at 55°C. for 30 minutes in order to disperse the acetate. Then, 20.00 ml. of 0.5 *N* NaOH is added from a burette and the solution is again heated at 55°C. for 30 minutes. The solution is then back-titrated with 0.5 *N* HCl.

A blank containing 20 ml. of pyridine and 20 ml. of 0.5 *N* NaOH is treated in similar fashion.

From the ml. of 0.5 *N* NaOH consumed in the digestion of the acetate less the correction found for the blank, the percentage of acetyl groups in the sample is calculated as follows:

$$\frac{(\text{ml. } 0.5\ N\ \text{NaOH} \pm \text{ml. for blank})\ (0.0215)\ (100)}{(\text{dry weight of sample, grams})} = \%\ \text{acetyl}$$

For samples which contain acid groups as well as ester groups, a preliminary titration (or separate determination) may be made in pyridine dispersion before the saponification step. Thus, for example, half esters of dibasic acids would be expected to exhibit half the acid value by titration alone as would be given in a simple saponification and titration procedure and accordingly, a preliminary titration before saponification permits estimation of half ester groups.

A microdetermination of acetyl groups has been developed for glycosides by Clark and Christensen (57).

✓(2) *Ether Groups.* These are probably the most difficult groups, as a class, to determine in a starch derivative. In common with other ethers they are relatively stable to acid and alkali and are not readily split from the carbohydrate and made available for determination. With simplest starch ethers such as the methoxyl derivatives, which have been extensively studied in fundamental research, it is possible to hydrolyze the carbohydrate chain completely to methyl glucoses and to recover these sugar derivatives with substantially all of the original substituent groups remaining intact. The application of these reactions to the study of starch structure has been discussed at some length in Chapter VIII.

The lower alkoxyl groups may be determined by a micro method as follows: 20.0 mg. of sample is boiled with 2.5 ml. of melted phenol and 5 ml. of HI in a suitable distillation equipment (58-59) through which CO_2 is passed continuously. The distillate is passed through an efficient absorption chamber into 5 ml. of a 10% potassium acetate solution in glacial acetic acid to which has been added 0.2 ml. of bromine. After a heating period of 60 minutes the collected distillate solution is washed into a 250 ml. flask with 5 ml. of a 25% aqueous solution of sodium acetate and the volume made up to 100 ml. The excess bromine is reduced with about 15 drops of formic acid. After removal of bromine, 0.5 g. of KI and 5 ml. of 10% H_2SO_4 are added and the liberated iodine is titrated with 0.05 N thiosulfate solution, using starch as an indicator. A blank is run on the reagents and this value is subtracted from the quantity of thiosulfate solution used when the sample was present.

Calculations for the two lowest alkoxyl groups are as follows:

$$\frac{(\text{ml. of } 0.05\ N \text{ thiosulfate for sample} - \text{blank})\ (25.86)}{(\text{weight of sample, dry basis, in mgs.})} = \%\ \text{methoxyl}$$

$$\frac{(\text{ml. of } 0.05\ N \text{ thiosulfate for sample} - \text{blank } 37.54)}{(\text{weight of sample, dry basis, in mgs.})} = \%\ \text{ethoxyl}$$

The more complex ethers do not lend themselves so readily to determination by the above method. However, several less direct methods are available and one or more of these may be found to apply to particular cases. First of all, if the substituent group of the starch ether contains some additional grouping which is readily determined than the ether may be analyzed by a quantitative measurement of this additional grouping. Two common examples are given which illustrate the general procedure.

✓*Carboxymethyl starch ethers.* These contain a carboxyl group on the ether side chains which may be determined by a procedure which amounts to a titration. Ordinarily these products are in the form of sodium salts. After combustion sodium carbonate remains. Titration of the ash permits calculation of the acid equivalent of the carboxymethyl ether. One gram of the product, dry basis, is ashed in a platinum dish at 600°C. Ashing is usually complete in 2 hours. After cooling, the ash is washed with 50 ml. of water into a 250 ml. flask, a drop of methyl orange is added and the solution titrated for sodium carbonate with 0.1 N HCl. From the amount of sodium ion found by titration the equivalent number of carboxyl groups in the sample is calculated and this is taken to be equal to the number of carboxymethyl groups. Since the ratio of sodium to starch at 1.0 D.S. carboxymethyl is 23/230, D.S. is calculated as:

$$\text{D.S.} = \frac{230}{23} \times \frac{A}{1.000} = 10\ A$$

for a 1.000 g. sample and where A is the weight of sodium, in grams, found by titrating the ash. Similarly, at zero substitution, D.S. = 7 A. Finally, over the range of 0 to 1 D.S.,* D.S. = $7A/(1\text{-}3\ A)$.

Allyl starch. In the simple case of the allyl ethers of starch, the allyl group contains a double bond. This may be estimated by classical methods for determination of unsaturation by the addition of halogens. Boyd and Roach (60) have evaluated the various halogen addition reactions with particular reference to the analysis of allyl starch. The well known Wijs procedure was found to give the most consistent results and was, at the same time, the simplest and most rapid. The iodine solution is prepared by dissolving 13 g. of resublimed iodine in 1 liter of glacial acetic acid and passing into this solution dried chlorine gas until the original thiosulfate titration value is almost doubled. A weighed amount of allyl starch is mixed with 15-20 ml. of chloroform and thoroughly dispersed. Then 25 ml. of the iodine solution is added. The reaction is allowed to take place in a glass stoppered bottle which is placed in a constant temperature bath at 25°C., in the dark, for 30 minutes. The stopper should be moistened with 15% potassium iodide solution to prevent loss of iodine or chlorine. Then 20 ml. of 15% potassium iodide and 100 ml. of water are added. The solution is titrated with 0.1 *N* thiosulfate solution, shaking the flask continually, until the yellow color of the solution has almost disappeared. Then starch indicator is added and the titration is continued until the blue color of the starch iodine complex has entirely disappeared. The flask should be shaken rather violently towards the end of the titration. A blank should be run under identical conditions. The number of moles of iodine taken up may be calculated from the difference between the two thiosulfate titration values, and from this the number of double bonds saturated may be estimated. It is assumed that the number of double bonds is equal to the number of allyl groups in the sample.

---

*Over the commercial range for this starch, the molar weight of a monomer unit increases from 162 at 0 D.S. to 230 at 1 D.S.

✓*Hydroxyl groups.* A determination of the hydroxyl groups in starch is sometimes employed as an indirect method for estimating the extent to which a product has been derivatized, as for example by etherification. The general procedure is to treat the starch with a known amount of acetylating agent, allow the free hydroxyl groups to become esterified and to determine the amount of acetylating agent which was consumed in the reaction.

A weighed quantity of sample, 1-3 g., is dispersed in exactly 15 ml. of a 20% by volume solution of acetic anhydride in pyridine. The reaction is allowed to proceed in a glass stoppered bottle which is heated on a steam bath. From 2 to 24 hours may be required to complete the reaction. The bottle is then cooled and 50 ml. of water is added to decompose the unreacted acetic anhydride into acetic acid. The acid is titrated with 0.6 *N* NaOH, using *o*-cresolphthalein as an indicator. A blank is run on 15 ml. of the acetic anhydride-pyridine solution. The per cent hydroxyl is calculated as follows:

$$\%\text{ hydroxyl} = \frac{(\text{ml NaOH for blank} - \text{ml NaOH for sample})\,(17)\,(\text{normality NaOH})}{10\ (\text{weight of sample, grams})}$$

The per cent hydroxyl may be used to calculate the D.S. of an ether in a manner indicated for starch methyl ethers in the example below. When the D.S. is zero, then there are, on the average, 3 hydroxyls on each monomer unit with a molar weight of 162 and the per cent hydroxyl is 3(17)/162 multiplied by 100 or 5100/162 which equals 31.5%. When the D.S. is 1, then there are 2 hydroxyl groups on each monomer unit with a molar weight of 192 and the per cent hydroxyl is 2(17)/192 multiplied by 100 or 3400/192 which equals 17.7%. Similarly, when the D.S. of the methyl ether is 2 then there is one hydroxyl group on a monomer with a molar weight of 222 and the per cent hydroxyl is 7.66. Of course when the D.S. is 3, the per cent hydroxyl is zero. From these four sets of values a curve is made, plotting % hydroxyl against D.S. of the starch ether. See also (61).

In some cases it may be preferable to determine the D.S. of an ether by this indirect method. For example, allyl starch ethers on aging or even often in preparation, undergo a secondary reaction through oxidation, polymerization, or both, which results in a loss in double bond unsaturation. An iodine or bromine value may, accordingly, give a result for D.S. of the starch product which is too low. However, if D.S. is estimated by a determination of the underivatized hydroxyls, a more accurate idea may be gained regarding the extent of substitution in the starch sample.

Naturally, when the substituent group contains a hydroxyl group, such as the hydroxy alkyl ethers, a determination of hydroxyl would not be a suitable means for determining D.S.

✓ *(3) Carboxyl Groups.* Carboxyl groups may be created in the starch molecule by oxidation which converts aldehydic and primary alcohol groups to acids. Occasionally, secondary alcoholic groups are so converted in extensive oxidations. Carboxyl groups may be introduced also on substituent groups in etherification such as the carboxymethyl derivatives discussed above. In many commercial grades of oxidized starches the number of carboxyl groups present on the starch chain may be very small and an ordinary titration with alkali would not be a suitable method for their determination. In such cases the carboxyl groups may be determined from the amount of acid liberated by cation exchange when the mineral-ion free material is treated with a solution of calcium acetate. The liberated acid is titrated with standard alkali after equilibrium has been reached. The general procedure, introduced into cellulose chemistry by Lüdtke (62) has recently been discussed and reviewed in this connection by Davidson and Nevell (63).

One gram of acid-washed, or otherwise demineralized material is placed in a 100 ml. conical flask with a ground glass stopper and 50 ml. of 0.05 *M* calcium acetate solution is added. The flask is shaken over night. The contents are centrifuged and 25 ml. of the clear solution pipetted into a 50 ml. Nessler tube. This solution is titrated with 0.01 *N* NaOH (carbonate free) to pH 8.5. For this titration, 0.5 ml. of a mixed indicator may be used which consists of 1 volume of 0.02% cresol red and 2 volumes of 0.02% thymol blue. This indicator mixture changes from pink to violet between pH 8.4 and 8.6.

Calcium acetate solution, as freshly prepared, has a pH value between 6.5 and 6.7. Exposure to air lowers the pH and if the value drops to 6.2, or below, for this or other reasons, an error may be introduced in the determination of carboxyl groups by this method.

A blank determination is run on the reagents and a correction is made accordingly.

It is assumed that the acid measured in the titration of the 25 ml. aliquot and multiplied by 2, is equivalent to the carboxyl groups present in the 1 g. sample.

✓*(4) Carbonyl Groups.* These are also introduced into the molecular structure of starch during certain oxidation reactions. They arise from secondary alcohol groups, for example, when starch is oxidized by periodic acid, a reaction which has been discussed at some length in Chapter XI. They are also formed to some extent in oxidations employed in the manufacture of commercial products.

Krajcinovic (64) has applied procedures which have been described for the determination of ketonic groups in oxycellulose to the analysis of oxidized starch. The determination is as follows: To 1 g. of oxidized starch, 20 ml. of a 0.1 *N* solution of hydroxylamine hydrochloride and 10 ml. of 0.1 *N* NaOH are added. About 20 minutes are allowed at room temperature for the reaction to be completed when freshly prepared reagents are used. The excess of unused hydroxylamine is determined by titration with 0.1 *N* HCl, using methyl orange as indicator. A blank determination is run for comparison. The carbonyl number is calculated according to the following equation:

$$\text{CO — number} = \frac{2.8\ (a - b)}{c}$$

in which *a* is the number of ml. of 0.1 *N* acid used to titrate the blank, *b*, the number of ml. of 0.1 *N* acid used to titrate the reaction mixture and *c*, the weight of oxidized starch in grams. In this manner the carbonyl number expresses in milligrams the amount of carbonyl groups in 1 g. of oxidized starch.

5. Carbamate, Carbanilate and Inorganic Acid Ester Groups. When groups are introduced into starch which contain atoms other than C, H, and

O, then naturally, the extent of derivatization may be determined from an analysis of the starch product for the additional element. Thus, for example, Wolff and Rist (65) determined the degree to which starch had been converted into a tricarbanilate by analyses for nitrogen and a comparison with the calculated percentage of nitrogen for starch tricarbanilate. In a similar manner, Caesar and Goldfrank (66) determined the degree of esterification in nitric acid esters of starch.

BIBLIOGRAPHY

1. Munson, L. S., and Walker, P. H., *J. Am. Chem. Soc.,* **28**, 663 (1906); Walker, P. H., *J. Am. Chem. Soc.,* **29**, 541 (1907).
2. Allihn, F., *Z. Ver. Ruebenzucker-Ind.,* 32 (N.F.19), 606, 685 (1882); see also, Bates, F. J., Polarimetry, Saccharimetry and the Sugars, Washington, D. C., 173 (1942).
3. *J. Assoc. Offic. Agr. Chemists,* **12**, 28 (1929); 18, 83 (1935); Foote, H. W., and Vance, J. E., *J. Am. Chem. Soc.,* **57**, 845 (1935); Fowler, R. M., and Bright, H. A., *J. Research Natl. Bur. Standards,* **15**, 493 (1935); Bright, H. A., *J. Research Natl. Bureau Standards,* **19**, 691 (1937).
4. McRary, W. L., and Slattery, M. C., *Arch. Biochem.,* **6**, 151 (1945); Assoc. of Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., A.O.A.C., Washington, D. C., 361 (1940).
5. Sumner, J. B., *J. Biol. Chem.,* **62**, 287 (1925).
6. Dreywood, R., *Ind. Eng. Chem., Anal. Ed.,* **18**, 499 (1946); Morris, D. L., *Science,* **107**, 254 (1948); Benham, G. H., and Despaul, J. E., *Anal. Chem.,* **20**, 933 (1948); Hestrin, S., and Mager, J., *Anal. Chem.,* **19**, 1032 (1947); Holzman, G., MacAllister, R. V., and Niemann, C., *J. Biol. Chem.,* **171**, 27 (1947).
7. Assoc. of Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., A.O.A.C., Washington, D. C., 359 (1940).
8. Nielsen, J. P., and Gleason, P. C., *Ind. Eng. Chem., Anal. Ed.,* **17**, 131 (1945).
9. Clendenning, K. A., *Can. J. Research,* **20**, 403 (1942); **B23**, 113 (1945); Clendenning, K. A., and Wright, D. E., *Can. J. Research,* **B23**, 131 (1945); Clendenning, K. A., *Can. J. Research,* **B23**, 239 (1945); **F26**, 185 (1948).
10. Earle, F. R., and Milner, R. T., *Cereal Chem.,* **21**, 567 (1944).
11. Hoffpauir, C. L., and Guthrie, J. D., *J. Am. Chem. Soc.,* **67**, 1225 (1945).
12. Steiner, E. T., and Guthrie, J. D., *Ind. Eng. Chem., Anal. Ed.,* **16**, 736 (1944).
13. Kerr, R. W., and Trubell, O. R., *Paper Trade J.,* **117**, No. 15, 25 (1943).
14. Horan, F. E., and Heider, M. F., *Cereal Chem.,* **23**, 492 (1946).
15. Hixon, R. M., Iowa Corn Research Institute, Ames, Iowa, 77 (1945).
16. Rask, O. S., *J. Assoc. Offic. Agr. Chemists,* **30**, 367 (1947).
17. Watson, S. A., and Whistler, R. L., *Ind. Eng. Chem., Anal. Ed.,* **18**, 75 (1946).
18. Pucher, G. W., Leavenworth, C. S., and Vickery, H. B., *Anal. Chem.,* **20**, 850 (1948).
19. Weinmann, H., *Plant Physiol.,* **22**, 279 (1947).
20. Van Voorst, F. Th., *Chem. Weekblad,* **39**, 468 (1942).
21. Baker, J. C., Parker, H. K., and Mize, M. D., *Cereal Chem.,* **20**, 267 (1943).
22. Schmidt-Nielson, S., and Hammer, L., *Kgl. Norske Videnskab, Selskabs, Forh.,* **5**, 84 (1932).
23. Samec, M., Kolloidchemie der Stärke, Dresden (1927).

24. Sair, L., and Fetzer, W. R., *Ind. Eng. Chem., Anal. Ed.,* **14**, 843 (1942).
25. Seeborg, E. F., *Amer. Assoc. Cereal Chem.,* **5**, 19 (1947).
26. Fosnot, R. H., and Haman, R. W., *Cereal Chem.,* **22**, 41 (1945).
27. Johnson, C. M., *Ind. Eng. Chem., Anal. Ed.,* **17**, 312 (1945).
28. Groves, L. G., and King, J., *J. Soc. Chem. Ind.* (London), **65**, 320 (1946).
29. Brockelsby, C. F., *J. Sci. Instruments,* **22**, 243 (1945).
30. Schroeder, C. W., and Nair, J. H., *Anal. Chem.,* **20**, 452 (1948).
31. Horan, F. E., Dissertation, Columbia University, New York (1944).
32. Swanson, M. A., *J. Biol. Chem.,* **172**, 805 (1948).
33. Höppler, F., *Chem. Ztg.,* **67**, 72 (1943).
34. Lansky, S., Kooi, M., and Schoch, T. J., Properties of the Fractions and Linear Subfractions from Various Starches, in press.
35. Meyer, K. H., Bernfeld, P., and Wolff, E., *Helv. Chim. Acta,* **23**, 854 (1940).
36. Staudinger, H., *Naturwissenschaften,* **25**, 673 (1937).
37. Caesar, G. V., Gruenhut, N. S., and Cushing, M. L., *J. Am. Chem. Soc.,* **69**, 617 (1947).
38. Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.,* **66**, 557 (1944).
39. Dombrow, B. A., Dissertation, Columbia University, New York (1944).
40. Bates, F. L., French, D., and Rundle, R. E., *J. Am. Chem. Soc.,* **65**, 142 (1943).
41. Dube, H. A., Dissertation, Iowa State College, Ames, Iowa (1947).
42. Kerr, R. W., and Schink, N. F., *Paper Trade J.,* **120**, No. 8, 77 (1945).
43. McCready, R. M., and Hassid, W. Z., *J. Am. Chem. Soc.,* **65**, 1154 (1943).
44. Baldwin, R. R., Bear, R. S., and Rundle, R. E., *J. Am. Chem. Soc.,* **66**, 111 (1944).
45. Kerr, R. W, and Severson, G. M., *J. Am. Chem. Soc.,* **65**, 193 (1943).
46. Kerr, R. W., *Arch. Biochem.,* **7**, 377 (1945).
47. Schoch, T. J., and Jensen, C. C., *Ind. Eng. Chem., Anal. Ed.,* **12**, 531 (1940).
48. Richardson, W. A., Higginbotham, R. S., and Farrow, F. D., *J. Textile Inst.,* **27**, 131 (1936).
49. Meyer, K. H., Wertheim, M., and Bernfeld, P., *Helv. Chim. Acta,* **23**, 865 (1940).
50. Levine, M., Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.,* **64**, 2331 1942).
51. Meyer, K. H., Noelting, G., and Bernfeld, P., *Helv. Chim. Acta,* **31**, 103 (1948).
52. Gore, H. C., and Steele, H. K., *Ind. Eng. Chem., Anal. Ed.,* **7**, 324 (1935).
53. Kerr, R. W., Trubell, O. R., and Severson, G. M., *Cereal Chem.,* **19**, 64 (1942).
54. Cleveland, F. C., and Kerr, R. W., *Cereal Chem.,* **25**, 133 (1948).
55. Kerr, R. W., and Cleveland, F. C., The molecular weight of the beta-amylase limit dextrin from corn starch, in press.
56. Murray, T. F., Staud, C. J., and Gray, H. L., *Ind. Eng. Chem., Anal. Ed.,* **3**, 269 (1931).
57. Clarke, R. A., and Christensen, B. E., *Ind. Eng. Chem., Anal. Ed.,* **17**, 334 (1945)
58. Houghton, A. A., *Analyst,* **69**, 363 (1944)
59. Assoc. of Official Agr. Chem., Official and Tentative Methods of Analysis, 5th ed., A.O.A.C., Washington, D. C., 647 (1940).
60. Boyd, H. M., and Roach, J. R., *Anal. Chem.,* **19**, 158 (1947).
61. Christensen, B. E., and Clarke, R. A., *Ind. Eng. Chem., Anal. Ed.,* **17**, 265 (1945).
62. Lüdtke, M., *Biochem. Z.,* **268**, 372 (1934); 285, 78 (1936).
63. Davidson, G. F., and Nevell, T. P., *J. Textile Inst.,* **39**, T102 (1948).
64. Krajcinovic, M., *Experientia,* **4**, 271 (1948).
65. Wolff, I. A., and Rist, C. E., *J. Am. Chem. Soc.,* **70**, 2799 (1948).
66. Caesar, G. V., and Goldfrank, M., *J. Am. Chem. Soc.,* **68**, 372 (1946).

Author Index

A

Adalberto, F., 323 (G, a)
Adams, B. A., 405 (55, 56)
Adams, F. H., 588 (16)
Adams, G. A., 115 (13, 14, 20), 533 (93, 96, 97, 98, 99, 103)
Adams, M., 438 (2)
Adams, M. H., 355 (1)
Adams, R., 321 (247)
Adams, S. L., 441 (123), 532 (33)
Adkins, H., 477 (107), 478 (117)
Ahlborg, K., 242 (97, 106)
Alexander, J., 86 (92), 153 (25)
Alexander, T. L., 115 (11)
Alfin, R. B., 439 (56)
Allihn, F., 689 (2)
Alsberg, C. L., 17 (9), 152 (5), 153 (37), 566 (13)
Amick, C. A., 657 (107)
Amthor, O., 373 (60)
Anchors, G. R., 314 (1)
Anderson, A. P., 86 (88, 89, 90)
Anderson, D. E., 531 (13)
Anderson, J. A., 534 (133)
Anderson, W. S., 116 (45)
Andes, R. V., 656 (70)
Andreasen, A. A., 441 (123), 532 (33)
Andrews, A. C., 567 (26)
Andrews, L. W., 476 (23)
Andrianov, K. A., 318 (155)
Anker, C. A., 152 (15)
Armstrong, E. F., 343 (36)
Armstrong, K. F., 343 (36)
Arzberger, C. F., 532 (53)
Asboth, A. von., 314 (3)
Ashe, L. H., 534 (132)
Ashford, W. R., 655 (32)
Ashford, W. R., 322 (D, a, b, c)
Ashworth, A., 314 (4)
Atkin, L., 441 (129)
Atkinson, R. W., 438 (8)
Averill, F. J., 241 (76)
Axtmeyer, J. H., 17 (1)

B

Back, T. M., 438 (12), 532 (34)
Badenhuizen, N. P., 17 (6, 7, 20, 23)
Badollet, M. S., 404 (40, 41)
Bailey, C. H., 566 (7)
Baird, K. D., 241 (53), 317 (100), 343 (41)
Bak, A. Z., 438 (30), 439 (43), 453 (3)
Baker, G., 153 (22)
Baker, J. C., 240 (19), 243 (166), 320 (208), 567 (25), 689 (21)
Baker, R. D., 60 (50)
Baker, W., 322 (E, a), 343 (47)
Balankura, B., 441 (123), 532 (33)
Balch, R. T., 116 (32, 35, 36)
Baldwin, M. E., 240 (20)
Baldwin, R. R., 178 (34, 35, 36), 240 (46), 243 (148, 151), 321 (A, a), 476 (40, 45, 49), 690 (44)
Balle, C., 322 (A, n)
Ballot, B., 314 (5)
Balls, A. K., 241 (93), 439 (68, 69, 70), 566 (18, 20)
Bamberger, M., 314 (6)
Bancroft, W. D., 626 (4)
Barab, J., 315 (40)
Barham, H. N., 116 (47, 48), 152 (16), 314 (7), 355 (3), 322 (C, a)
Barker, C. C., 241 (67)
Barlow, J. J., 373 (31)
Barnett, W. L., 314 (8)
Bartling, F. W., 60 (7, 10, 20), 405 (69)
Bartman, L., 60 (26)
Bartovics, A., 243 (158)
Bates, F. J., 689 (2)
Bates, F. L., 178 (30), 240 (28), 476 (42), 690 (40)
Battestin, M., 242 (113)
Bauer, H. F., 86 (60, 77), 372 (7), 477 (93), 626 (18, 19, 20, 25)
Bauer, J. V., 85 (20), 372 (7), 477 (93), 512 (3), 626 (17, 19, 20, 21)
Bauer, L. P., 61 (83, 84)
Bauman, W. C., 405 (63)
Baur, F. J., Jr., 60 (12)
Bawn, C. E. H., 242 (124)
Bayer, F., 314 (9)
Beadle, C., 315 (43, 44)
Bear, R. S., 177 (11, 15, 23, 29), 178 (34), 240 (37, 46), 244 (208), 321 (A, a, b, c), 476 (45, 51), 690 (44)
Beati, E. B., 477 (109), 478 (112, 113)

Béchamp, A., 314 (10), 323 (G, b)
Becher, W., 84 (5)
Bechtel, W. G., 152 (17, 18, 19)
Beck, F., 314 (12)
Beckmann, C. O., 178 (41, 45), 243 (163)
Beckord, L. D., 439 (49, 50), 441 (131, 132), 532 (25, 26)
Beckord, O. C., 153 (42), 439 (42)
Behrman, A. S., 405 (60)
Bell, A. C., 152 (8)
Bell, D. J., 241 (59, 78, 89)
Bemis, W. A., 405 (73)
Benham, G. H., 689 (6)
Benson, A. A., 244 (223)
Berczeller, L., 476 (31)
Berentzen, H., 85 (45)
Beresford, H., 453 (17), 531 (18)
Berg, J. C., 315 (22)
Bergen, W. S., 534 (113)
Bergh, F. P., 314 (11)
Bergmann, M., 314 (12, 13, 14)
Bergquist, C., 60 (25), 318 (133), 343 (52), 373 (23), 477 (98)
Berl, E., 314 (15, 16, 17, 18), 655 (34)
Berlin, H., 61 (79), 372 (16), 404 (9, 22)
Bernfeld, P., 17 (17), 177 (25), 178 (33), 240 (21, 22, 23, 44), 241 (94), 242 (95, 112, 120, 122), 318 (160a), 323 (G, k), 439 (53, 54, 55, 63, 64, 65, 66, 67), 440 (110, 111), 476 (56), 690 (35, 49, 51)
Berrigan, J. L., 60 (34)
Berthelot, 315 (19)
Berthon, P., 315 (20)
Beutel, E., 321 (244)
Bevan, E. J., 315 (43, 44, 45, 46)
Beyer, G. F., 533 (65)
Bice, C. W., 17 (14), 115 (12), 655 (11, 12)
Biedermann, W., 85 (38, 39, 40, 41, 42)
Bikerman, J. J., 626 (6, 13)
Billing, W. M., 86 (64)
Bingham, E. C., 153 (27)
Bingham, R. T., 513 (42)
Bishop, J. P., 60 (24)
Black, W. C., 512 (5)
Blair, J. S., 626 (27)
Blaser, B., 321 (249)
Bley, R. S., 322 (A, d)
Bleyer, B., 404 (17)
Blinc, M., 240 (13), 320 (210), 342 (23)
Blish, M. J., 60 (23), 439 (35, 73), 441 (146), 453 (2, 4), 566 (2, 4, 8, 9), 567 (30)
Bloede, V. G., 85 (32), 372 (5), 373 (20)
Blom, J., 438 (30), 439 (43, 46), 453 (3)
Blom, R. H., 441 (125), 534 (109), 655 (9)
Blondeau, C., 315 (21)
Blumer, E., 477 (97)
Bock, L., 512 (37)
Bode, C. E., 566 (4)
Bode, H. E., 372 (15)
Boeseken, J., 315 (22)
Boettinger, C., 315 (23)
Boidin, A., 441 (127, 128)
Boie, H., 61 (67)
Bois, E., 440 (106)
Bolz, F., 318 (161), 404 (14)
Bond, D. C., 656 (53, 55)
Booher, L. E., 439 (62)
Booth, R. B., 656 (59)
Boppel, H., 241 (62, 63, 64), 316 (76)
Borglin, J. N., 655 (21)
Boruff, C. S., 531 (3)
Boswell, V. R., 116 (45)
Bottazi, F., 315 (39)
Bourne, B. A., 116 (41)
Bourne, E. J., 438 (11), 441 (142, 143, 144)
Bourne, J. E., 178 (31), 244 (209, 210, 211)
Bower, R. S., 241 (90)
Boyd, H. M., 690 (60)
Boyd, R. N., 320 (228), 405 (59)
Braae, B., 439 (43)
Braconnot, H., 315 (24)
Brand, R., 534 (116)
Brandon, B. A., 477 (78)
Breden, C. R., 533 (81)
Brautlecht, C. A., 116 (24)
Bredt, O., 320 (225)
Brenchley, W. E., 17 (12)
Brender, C., 322 (E, b)
Bretano, W., 177 (25), 240 (21)
Brewster, J. F., 477 (86)

Bridgman, W. B., 178 (40)
Briggs, D. R., 243 (169)
Briggs, J. F., 315 (45)
Bright, H. A., 689 (3)
Brigl, P., 315 (25)
Brimhall, B., 17 (3), 60 (9), 116 (51), 153 (26, 32), 315 (26), 323 (G, x), 372 (2)
Brindle, R. G., 60 (36), 373 (56)
Brock, F. H., 405 (79)
Brockelsby, C. F., 690 (29)
Brooks, D. B., 531 (14)
Broseff, W., 60 (14)
Brown, C. J., 534 (127)
Brown, E. H., 656 (74, 75, 76)
Brown, F., 241 (79), 243 (143)
Brown, F. R., 626 (1)
Brown, H. T., 177 (7), 315 (27), 441 (145), 566 (10)
Brown, J. B., 60 (12)
Brown, J. M., 405 (73)
Brown, K. R., 655 (22)
Brown, L. T., 531 (9)
Brown, R. W., 656 (71)
Brown, W. R., 315 (28), 342 (16)
Browning, B. L., 476 (46)
Browning, W., 373 (31)
Bruce, T. A., 373 (41, 42)
Bruins, P., 534 (113)
Bruson, H. A., 315 (29)
Bryant, A. P., 85 (19), 322 (E, c), 343 (50)
Bryner, L. C., 532 (56)
Buchanan, M. A., 404 (27)
Buchler, C. C., 316 (88)
Buel, H., 153 (21)
Bütler, R., 314 (15, 16)
Bulfer, A. J., 85 (14), 373 (18)
Bumann, I., 178 (38)
Bungenberg de Jong, H. G., 85 (48), 244 (191)
Bunnell, L. A., 655 (1)
Bunting, A. H., 244 (221)
Burger, E. K., 531 (2)
Burkhard, C. A., 315 (30, 31, 32), 323 (G, c)
Buswell, A. M., 258 (6)

C

Caesar, G. V., 85 (54), 152 (12), 258 (5), 315 (33), 322 (A, f), 355 (2, 6, 9, 10, 11), 373 (47), 477 (90), 626 (23), 655 (38), 690 (37, 66)
Cake, W. E., 477 (106)
Caldwell, C. G., 116 (53), 241 (80), 243 (138), 316 (89), 317 (124)
Caldwell, M. J., 314 (7), 322 (C, a), 439 (62)
Caldwell, M. L., 438 (2), 439 (45, 56, 62), 440 (90, 91, 108, 109)
Callaghan, R. W., 115 (16, 18)
Calvin, M., 244 (223)
Campbell, G. F., 439 (61)
Cannon, G. E., 656 (66, 67)
Cantor, S. M., 239 (1), 317 (119), 404 (16, 18, 31, 33), 405 (62, 90)
Carey, C. H., 655 (41)
Carney, D. M., 241 (86)
Carolles, B., 315 (34)
Carpenter, C. B., 656 (72)
Carter, R. E., 512 (39)
Carter, S. R., 241 (61)
Casey, J. P., 512 (2, 9, 25), 513 (46)
Cattle, M., 439 (44), 476 (32)
Cavalieri, D. K., 404 (45)
Cavalieri, L. F., 404 (35, 45)
Cerf, L., 315 (35)
Chalmot, G. de, 315 (36)
Chowdhury, J. K., 315 (37)
Christensen, B. E., 690 (57, 61)
Christensen, L. M., 440 (120), 453 (17), 531 (2, 8, 11, 18, 19, 20), 532 (22, 23, 30, 38, 45, 47, 56, 57), 533 (82, 84)
Chrzaszcz, T., 152 (4), 439 (76, 77)
Church, M. B., 440 (117)
Church, R. J., 512 (39)
Claborn, H. V., 533 (71, 72, 73)
Clark, G. L., 626 (9)
Clark, H. T., 315 (38)
Clarke, R. A., 690 (57, 61)
Classen, A., 477 (73, 94, 95), 655 (5, 6)
Clayson, D. H. F., 244 (185)
Clegg, J. E., 373 (46)
Clemmer, J. B., 656 (73, 77)
Clemmons, B. H., 656 (73, 77)

Clendenning, K. A., 115 (15), 405 (74), 534 (118, 119), 689 (9)
Cleveland, F. C., 240 (31, 40, 42), 439 (51), 690 (54, 55)
Cobb, R. M., 512 (7)
Cochran, H. L., 116 (45)
Cocking, A. T., 533 (63)
Coehn, A., 315 (39)
Coghill, R. D., 533 (87, 88), 534 (117, 126)
Cole, A. T., 656 (78)
Coleman, G. H., 241 (83), 404 (27)
Coles, J. S., 178 (42)
Collatz, F. A., 566 (12)
Colowick, S. P., 244 (215, 216, 217, 218)
Colwell, A. T., 531 (13)
Conaway, R. F., 512 (36)
Connor, R., 477 (107), 478 (115)
Connstein, W., 532 (62)
Cook, D. H., 17 (1)
Cooke, L. M., 322 (D, a)
Cooper, M., 477 (75)
Cope, W. C., 315 (40)
Copeland, E. B., 98 (2)
Coppock, P. D., 373 (45)
Cori, C. F., 177 (29), 178 (32), 244 (196, 199, 200, 203, 207, 208, 212, 215, 216, 217), 321 (A, c), 404 (8), 441 (134, 139, 140)
Cori, G. T., 244 (196, 199, 200, 203, 206, 207, 212, 216), 441 (134, 137, 138, 139, 140)
Corman, J., 242 (119), 438 (15), 440 (100), 441 (125)
Cornwell, R. T. K., 534 (106)
Covert, L. W., 477 (107)
Cox, M. J., 17 (21), 60 (16)
Craft, B. C., 656 (50, 51)
Craig, W. L., 512 (31)
Craik, J., 315 (41)
Cramer, F. B., 315 (42)
Crawford, F. M., 532 (43)
Cross, C. F., 315 (43, 44, 45, 46)
Cummings, R. E., 531 (13)
Curry, H. W., 61 (55)
Curtis, J. J., 60 (8)
Cushing, M. L., 258 (5), 355 (6, 9, 11), 690 (37)

D

Daasch, L., 177 (14), 323 (G, u)
Dale, J. K., 405 (77), 453 (20), 566 (16)
Daly, R. E., 84 (1)
Damansky, A. F., 315 (47, 48), 319 (199, 200)
Danielsson, C. E., 242 (121), 439 (71, 72)
Darrah, W. A., 373 (57)
Daumiller, G., 320 (230)
Davidson, G. F., 690 (63)
Davis, H. A., 115 (21), 372 (1)
Davis, U. R., 534 (105)
Davison, W. C., 531 (16)
Dax, P. J., 439 (40)
Day, H. G., 244 (204)
Dean, G. R., 404 (33)
de Bruyne, N. A., 626 (10)
DeCastro, J., 61 (58)
de Claubry, G., 475 (1)
De Clerck, J., 440 (114)
Degering, E. F., 315 (30, 31, 32, 49, 50, 50 a, 51), 318 (158), 319 (178), 322 (B, c), 323 (G, c), 533 (80), 657 (91)
Deitz, V. R., 405 (47)
Dejarme, N., 322 (A, g)
Derksen, J. C., 177 (13)
de Saussure, T., 403 (2)
Despaul, J. E., 689 (6)
DeTurk, E. E., 60 (11)
Deutsch, P. A., 567 (52)
Dhar, N. R., 315 (52), 319 (181, 182), 342 (11, 12, 13, 14, 15), 476 (30)
Di Carlo, F. J., 439 (74), 440 (84, 102)
Dickson, A. D., 439 (36, 37)
Diehm, R. A., 512 (22)
Dietz, A. A., 533 (80)
Dimler, R. J., 115 (19, 21), 372 (1)
Dinglinger, A., 318 (162)
Dloughy, J. E., 405 (72)
Dobry, A., 244 (195)
Dollfus, E., 320 (217)
Dombrow, B. A., 178 (45), 243 (161), 6[illegible] (39)
Donovan, P. P., 323 (G, s)
Dore, W. H., 241 (54), 317 (97)
Doree, C., 322 (A, h)
Doudoroff, M., 244 (225)
Dreby, E. C., 588 (13)

Dreher, E., 322 (A, o)
Dreyer, A., 373 (58)
Dreyfus, H., 315 (53), 323 (G, d)
Dreywood, R., 689 (6)
Dritter, F., 86 (84)
Dube, H. A., 243 (153), 476 (44), 690 (41)
Duckert, F., 439 (67)
Duel, H., 477 (87)
Dürr, W., 318 (161)
Duke, J. B., 656 (78)
Dulac, R., 626 (16)
Dumpert, G., 316 (79), 476 (34)
Dunn, T. H., 656 (60)
Dunning, J. W., 532 (59), 534 (110, 128)
Dunstan, S., 243 (143)
Du Pont, 315 (54)
Dupre, 98 (6)
Durieux, O., 315 (55), 342 (10)
Durr, W., 404 (14)
Duryea, C., 85 (29)
Duryea, C. B., 405 (49)

E

Earl, F. R., 60 (8)
Earle, F. R., 689 (10)
Early, E. B., 60 (11)
Ebert, C., 404 (11)
Eble, O. H., 84 (8)
Eckland, E., 60 (27)
Edsall, J. T., 477 (81)
Edson, E. R., 373 (36), 626 (18)
Edwards, D. W., 243 (164)
Edwards, F. C., 177 (18), 243 (150), 476 (39)
Effront, J., 440 (103), 441 (126, 127, 128)
Efron, A., 534 (109)
Ehrmann, M. P., 316 (85)
Ehrnst, L. E., 439 (39)
Eigeless, M. A., 656 (79)
Eilers, H., 320 (231)
Eissler, F., 319 (194)
Elder, A. L., 533 (86)
Elion, E., 567 (27)
Ellis-Anwyl, R. H., 655 (2)
Elssner, R., 315 (56)
Elverum, G. W., 115 (16)
Eoff, J. R., 533 (64, 65)
Epstein, S. F., 244 (222)
Erb, N. M., 441 (124), 532 (32, 55)
Ericks, W. P., 657 (99)
Erickson, J. A., 656 (80)
Ermen, W. F. A., 152 (3)
Escales, R., 315 (57)
Eva, W. J., 566 (3)
Evans, J. W., 86 (67)
Evans, R., 439 (36, 37)
Evans, R. B., 86 (59), 373 (54)
Evans, T. H., 322 (D, b), 655 (32)
Eveking, W., 317 (106 a)
Everett, M. R., 316 (58), 343 (27)
Eynon, L., 115 (3)
Eyring, H., 258 (7, 9)
Eyster, H. C., 440 (83)

F

Farley, F. F., 241 (86), 316 (65, 67), 342 (24, 26)
Farrow, F. D., 85 (30), 153 (29), 241 (55), 243 (147), 323 (E, t), 343 (37), 588 (8), 690 (48)
Favor, H. H., 86 (65), 567 (39)
Fehling, H., 316 (66), 323 (G, g)
Feigel, F., 60 (22)
Fein, M. L., 533 (74)
Fellers, C. R., 404 (43)
Felske, F. G., 512 (33)
Felton, G. E., 84 (12), 116 (52), 316 (67), 323 (E, u), 342 (24), 343 (58)
Feltzer, A., 60 (37)
Ferdman, D. L., 244 (222)
Fernbach, A., 316 (68), 323 (F, a), 342 (9)
Fetzer, W. R., 153 (45), 405 (83), 690 (24)
Field, F. B., 322 (E, a)
Field, F. R., 343 (47)
Fielding, A., 373 (32, 33)
Filachione, E. M., 533 (77)
Filley, H. C., 531 (11)
Fioroni, W., 317 (127)
Fischer, E. H., 241 (94), 439 (63, 64, 65, 67), 440 (110, 111)
Fischer, E. K., 153 (35)
Fischer, K., 318 (162)
Fisher, C. H., 533 (74, 75, 76, 77)
Fiske, A. H., 85 (16)

Fletcher, H. H., 355 (1)
Fleury, P., 242 (133), 317 (104), 343 (30)
Flexser, L. A., 478 (111)
Flory, P. J., 477 (84)
Fodor, A., 438 (16)
Foote, H. W., 689 (3)
Ford, C. L., 116 (52)
Ford, J. S., 566 (5)
Ford, W. P., 566 (21)
Fordyce, C. R., 316 (69)
Fortmann, K., 566 (23)
Fosnot, R. H., 690 (26)
Fosse, R., 316 (70, 71)
Foster, J. F., 178 (35, 44, 46), 241 (81), 243 (151, 154), 316 (72), 318 (141), 342 (19), 403 (4), 476 (43, 49), 690 (38, 50)
Foster, J. L., 656 (56)
Fouard, E., 323 (F, b)
Fowler, A. M., 689 (3)
Fowler, G. B., 477 (104), 512 (17)
Fox, T. G., 243 (163)
Fraenkel-Conrat, H. L., 477 (75, 76, 77, 78, 79, 80)
Frahm, H., 372 (1)
Franchimont, A., 316 (73)
Frank, A., 98 (8)
Frank, G., 322 (B, a), 323 (G, m)
Frankenfield, F. A., 84 (1)
Fred, E. B., 532 (48), 534 (133)
French, A. H., 86 (86)
French, D., 177 (8, 11, 14, 16, 21, 24), 240 (28, 29), 243 (149), 323 (G, v), 476 (37, 38, 42), 477 (81), 567 (38), 690 (40)
Frere, J., 316 (74)
Freudenberg, K., 177 (6), 178 (38), 241 (62, 63, 64, 65), 316 (75, 76, 77, 78, 79), 318 (161), 404 (13, 14), 476 (34)
Frey, C. N., 441 (129), 566 (1)
Frey-Wyssling, A., 17 (8), 177 (4)
Friedel, C., 316 (80)
Frieden, A., 626 (28)
Friedenthal, H., 476 (25)
Friedrich, K., 178 (38)
Friese, M., 316 (81)
Fritsche, F., 316 (82)
Frost, F. H., 512 (30)
Frush, H. L., 317 (121), 477 (86)
Fukushima, I., 316 (83)
Fuld, M., 17 (19), 240 (35), 242 (95), 439 (55, 66)
Fuller, A., 373 (37)
Fuller, A. D., 322 (E, d), 343 (53), 373 (24, 25, 26)
Fulmer, E. I., 440 (119, 121), 453 (14, 15, 16), 531 (1, 8), 532 (56, 57, 58), 533 (69, 70, 81, 82), 534 (128, 131, 135)
Furry, M. S., 588 (9)

G

Gabriel, C. L., 531 (2), 532 (42, 43)
Gailey, F. B., 534 (127)
Gale, R. A., 453 (12)
Gallay, W., 152 (8)
Gapen, C. C., 373 (18)
Gardner, H. F., 373 (61), 512 (33)
Gardner, T. S., 315 (42)
Garrison, M., 116 (45)
Gastrock, E. A., 534 (129)
Gates, R. L., 439 (47), 440 (122)
Gatin-Gruzewska, Z., 240 (9)
Gault, H., 316 (84, 85)
Gaver, K. M., 655 (23, 28, 29, 30, 43), 656 (87)
Geddes, W. F., 152 (15), 438 (4), 566 (3, 22), 567 (28, 32, 37), 567 (37)
Geller, H. H., 534 (111, 112)
Genin, G., 316 (86)
Genung, L. B., 316 (87)
Georges, L. W., 241 (90), 242 (116)
Georgiyesky, 567 (40)
Gerber, C., 316 (90)
Gessner, L., 319 (177)
Getty, J. E., 512 (33)
Giesecke, F. O., 60 (39), 86 (71, 93, 94, 95), 656 (82, 83, 84)
Gill, A., 532 (40)
Gill, L. O., 85 (22), 86 (81, 86), 588 (14)
Gillan, E. P., 512 (20)
Gillespie, H. B., 315 (38)
Gilman, J. C., 60 (18)
Giri, K. V., 440 (98, 99)
Glabe, E. F., 567 (54)

Gladding, E. K., 243 (142), 322 (E, e), 343 (39)
Glarum, S. N., 86 (61, 62), 373 (52, 53)
Glasstone, S., 258 (7, 9)
Gleason, P. C., 689 (8)
Goering, K. J., 440 (120), 532 (30, 31)
Goettsch, H. M., 476 (23)
Goikhman, N. G., 153 (43, 44), 567 (41, 42)
Goldberg, H., 153 (36)
Goldfrank, M., 315 (33), 355 (10), 655 (38), 690 (66)
Gomberg, M., 317 (88)
Gordon, K. M., 239 (2), 404 (19, 20)
Gordon, M., 17 (15)
Gore, H. C., 438 (29), 566 (6), 690 (52)
Gore, R. C., 258 (6), 440 (97)
Gottlieb, D., 316 (89)
Grace, N. H., 115 (14, 15)
Graesser, F. R., 439 (40)
Graham, T., 626 (2)
Grangaard, D. H., 243 (139, 142), 343 (39, 40), 322 (E, e, f)
Graumann, E., 320 (220)
Gray, G. R., 656 (57, 61)
Gray, H. L., 690 (56)
Green, A. A., 441 (137, 138, 139)
Green, H. G., 153 (33)
Greenfield, R. E., 60 (45)
Gregory, H., 322 (A, k)
Griffin, I. L., 85 (56, 57), 477 (89), 588 (15)
Groen, M. G., 322 (A, i), 655 (31)
Groggins, P. H., 531 (2)
Grove, O., 532 (27)
Groves, L. G., 690 (28)
Gruenhut, N. S., 355 (9), 690 (37)
Grunert, C., 321 (249)
Gruzewska, Z., 315 (39), 316 (90)
Guthrie, J. D., 689 (11, 12)
Guthrie, J. M., 566 (5)
Guymon, J. F., 534 (128)

H

Haage, H., 656 (88)
Haake, J. W., 86 (80)
Haake, R., 322 (E, g), 373 (48)
Haake, W., 316 (91), 322 (E, g), 373 (48)
Haberman, J., 316 (92), 342 (3)
Habrle, J. A., 177 (9)
Hackel, J., 319 (196)
Haehn, H., 85 (43, 45)
Haerdtl, H., 240 (10), 320 (208)
Haerz, E., 317 (93)
Hagedorn, M., 317 (94), 323 (A, j)
Hale, F. E., 475 (6)
Hale, W. J., 531 (7)
Hale, W. S., 566 (18, 20)
Halen, S., 317 (95)
Hall, H. E., 532 (54)
Hall, L., 657 (103)
Haller, 323 (E, s)
Haller, R., 316 (74), 322 (C, b)
Halsall, T. G., 242 (127), 243 (143, 144, 145), 343 (30)
Haman, R. W., 690 (26)
Hamilton, R. M., 317 (96), 319 (172, 173), 657 (98)
Hammer, L., 689 (22)
Hammerstein, F. L., 405 (82)
Hampel, C. A., 84 (9, 10)
Handelman, M., 405 (64)
Hanes, C. S., 177 (5), 242 (111), 244 (197, 198, 201, 202), 439 (44), 440 (89), 441 (135, 136), 453 (1), 476 (32, 33)
Hanig, M., 243 (169)
Hao, L. C., 440 (121), 453 (16)
Hansen, D. W., 60 (9), 343 (62), 657 (100)
Harada, T., 440 (118)
Harder, M., 316 (77)
Hardin, M. B., 116 (27)
Harrison, W., 318 (147), 323 (G, h, i, m), 475 (7, 15)
Harvey, E., 85 (34)
Harvey, E. H., 322 (E, h), 343 (55)
Hassid, W. Z., 177 (28), 240 (45), 241 (54, 70), 244 (206, 224, 225, 227), 317 (97), 476 (47), 690 (43)
Hatschek, E., 153 (30)
Hauser, E. A., 513 (44)
Hawkins, E. G. E., 317 (98)
Hawley, D. M., 372 (7), 477 (93), 626 (19, 20, 21)
Haworth, W. N., 239 (4, 6), 240 (39, 49, 50, 52), 241 (53, 60, 68, 69, 92), 244

(205, 209), 317 (99, 100, 101), 322 (A, k), 323 (G, j), 343 (38, 41), 403 (3), 441 (143)

Hayek, M., 405 (75)

Haywood, G., 512 (28)

Heald, A. M., 85 (28), 512 (6)

Heath, R. L., 241 (69), 244 (205)

Heider, M. F., 116 (46), 689 (14)

Heidt, L. J., 343 (39)

Helferich, B., 317 (102)

Helle, J., 320 (207), 655 (37)

Henry, W. F., 60 (24)

Herbert, D., 440 (104)

Hermite, F., 317 (105)

Heron, J., 566 (10)

Herrera, J. J., 316 (75)

Herrick, H. T., 533 (67)

Herrissey, H., 242 (133), 317 (104), 343 (30)

Herstein, B., 477 (100), 655 (7)

Hess, K., 240 (14, 15), 241 (72, 73, 82), 317 (106, 107, 108, 109)

Hestrin, S., 441 (141), 689 (6)

Heuer, W., 320 (229 b)

Hewett, P. S., 512 (39)

Heyne, K., 98 (5)

Hibbert, H., 322 (D, a, b, c, E, i), 343 (46)

Hickey, R. J., 533 (69, 70)

Hidy, P. H., 244 (204)

Higginbotham, R. S., 85 (30), 153 (20), 240 (48), 241 (55), 243 (147), 317 (110), 323 (E, t), 343 (37), 690 (48)

Hilbert, G., 476 (58)

Hilbert, G. E., 17 (14), 60 (16), 115 (12, 21), 116 (49), 240 (38), 242 (115), 320 (222), 321 (257, 259), 372 (1), 531 (12), 655 (12), 657 (93)

Hilbert, H., 655 (32)

Hildebrand, F. C., 439 (45)

Hildebrandt, F. M., 532 (32, 55)

Hildesheimer, A., 532 (60)

Hill, H. S., 343 .(46)

Hill, R., 533 (85)

Hiller, L. A., Jr., 242 (125), 404 (34)

Hilliard, F. C., 373 (46)

Hinton, J. J., 115 (10)

Hirst, E. L., 239 (6), 240 (51, 52), 241 (53, 60, 66, 67, 71), 242 (124, 127), 243 (144, 145), 317 (99, 100, 101, 111), 320 (233), 343 (30, 38, 41)

Hiss, H. S., 322 (E, i)

Hitchcock, A. S., 60 (5)

Hixon, R. M., 115 (6, 7), 116 (51), 153 (26, 32), 178 (44), 241 (80, 81, 87), 243 (138, 154), 244 (184), 315 (26), 316 (65, 72, 89), 317 (124), 318 (141), 323 (G, x), 342 (19, 24, 26), 403 (4), 476 (55), 531 (8, 9), 534 (135), 689 (15), 690 (38, 50)

Hjermstad, E. T., 513 (41)

Hobbs, K. C., 405 (90)

Hockenhull, D. J. D., 440 (104)

Hockett, R. C., 322 (E, j), 343 (44)

Hodge, J. E., 241 (75)

Hoefft, F. von, 320 (213)

Hoenig, M., 317 (112, 113, 114, 115)

Höppler, F., 85 (25), 152 (9), 316 (91), 655 (24), 690 (33)

Hoffpauir, C. L., 689 (11)

Hofman, E., 534 (130)

Hoggatt, G. H., 655 (3)

Hohenemser, W., 318 (160 a)

Holberger, J. H., 317 (116)

Holden, H. C., 60 (35)

Hollenbeck, C. M., 439 (73, 75), 453 (2 11)

Holmes, E. L., 405 (55, 56)

Holmes, F. B., 322 (D, d)

Holt, N. B., 477 (86)

Holzman, G., 689 (6)

Hopff, H., 318 (161)

Hopkins, D. G., 626 (5)

Hopkins, E. F., 116 (39, 40)

Hopkins, R. H., 438 (26), 440 (101, 112)

Hoppler, E. F., 86 (80)

Horan, F. E., 116 (46), 240 (47), 68 (14), 690 (31)

Horesi, A. C., 372 (14), 405 (70)

Houghton, A. A., 317 (117), 690 (58)

Houtz, C. C., 626 (15)

Howald, A. M., 512 (38)

Hubbard, J. E., 60 (8)

Hubert, P., 98 (6)

Hudson, C. S., 242 (134, 135, 136, 137), 317 (122, 123, 124), 343 (31, 32, 33, 35), 438 (31, 32)
Huggins, M. L., 243 (155, 156, 160)
Hughes, G. K., 317 (118)
Hurd, C. D., 239 (1, 2), 317 (119), 404 (18, 19, 20, 21)
Husemann, E., 241 (57, 58), 320 (232, 233), 655 (16)

I

Iddles, H. A., 243 (173)
Ikeda, C., 440 (81, 82)
Iler, R., 85 (55)
Iljin, W. S., 85 (44)
Inoue, R., 320 (207 a)
Ipatieff, W., 477 (105)
Irvine, J. C., 239 (5), 317 (120)
Isaacs, E., 533 (85)
Isbell, H. S., 317 (121), 477 (86)
Isherwood, F. A., 241 (60)

J

Jackson, E. L., 242 (134, 135, 136, 137), 317 (122, 123, 124), 343 (31, 32, 33, 35)
Jacobs, P. B., 531 (5)
Jacobs, W. L., 441 (124)
Jacoby, M., 477 (66)
Jaeger, C. M., 59 (4), 115 (9)
James, G. M., 244 (221)
James, W. O., 244 (221)
Janicki, J., 152 (4), 439 (76, 77)
Jeanes, A., 321 (258, 259)
Jeffries, F. L., 60 (33, 46, 47, 48, 49)
Jenemann, J. A., 533 (79)
Jensen, C. C., 240 (30), 355 (5), 690 (47)
Johnson, C. M., 690 (27)
Johnson, J. A., 567 (34)
Johnson, M. J., 534 (127)
Johnson, M. L., 532 (48)
Johnston, N. F., 86 (65), 567 (39)
Johnston, W. R., 438 (27)
Jolitz, C. E., 566 (9)
Joly, M., 242 (133), 317 (104), 343 (30)
Jones, J. K. N., 241 (79, 88), 242 (127), 243 (144, 145), 317 (98), 343 (30)
Jones, W. L., 373 (21)
Jordan, S., 567 (44)
Jorgensen, H., 566 (19)
Józsa, S., 438 (27, 29), 566 (6)
Jud, J., 655 (13)
Jurgensen, R. P., 61 (80), 405 (50)

K

Kabat, E. A., 243 (178)
Kagan, B. O., 244 (226)
Kalckar, H., 244 (214)
Kalinowsky, J. von, 317 (125)
Kantorowicz, J., 86 (82, 83), 323 (E, k), 343 (54)
Kaplan, N., 244 (225)
Kappesser, R., 655 (16)
Karow, E. O., 534 (121)
Karrer, P., 239 (3), 242 (132), 317 (126, 127, 128, 129), 318 (156), 343 (29), 655 (14)
Katayama, M., 476 (29)
Kattein, A., 476 (27)
Katz, J. R., 177 (12, 13), 567 (35), 588 (5, 6)
Katznelson, H., 533 (90, 91, 92, 100)
Katzbeck, W. J., 476 (62)
Kaulla, E. N., v., 655 (16)
Keitt, T. C., 116 (31)
Kelling, A. H., 61 (69, 70, 71, 72, 73, 74, 75)
Kendall, A. R., 533 (82)
Kent-Jones, D. W., 318 (133), 567 (29)
Kenyon, W. D., 242 (130)
Kenyon, W. O., 321 (253)
Kerb, J., 317 (130)
Keresztesy, J. C., 258 (3)
Kern, W., 316 (75)
Kerr, G. A., 61 (59)
Kerr, R. W., 60 (6, 15, 32, 51), 61 (52, 79), 84 (3), 85 (13, 15, 18, 21, 36), 116 (26), 152 (2, 6), 153 (34), 177 (2, 20), 240 (24, 25, 26, 27, 31, 40, 41, 42, 43), 242 (117, 118, 129), 244 (179, 189), 258 (11), 318 (131), 320 (222), 323 (E, l, m), 343 (51, 57), 355 (4), 373 (22, 50), 403 (5), 404 (32), 438 (33), 439 (34, 51, 57, 58), 453 (5, 6, 8, 10, 19), 476 (36, 48, 52, 53, 62), 477 (91), 512 (8, 10, 11, 15, 16, 19, 21, 29), 566 (15, 17), 567 (36,

43, 47, 49, 53), 588 (11, 17), 655 (17), 656 (68), 689 (13), 690 (42, 45, 46, 53, 54, 55)
Kerstjens, A. H., 315 (22)
Kesler, C. C., 86 (68, 75), 152 (17, 19), 512 (5, 33), 513 (41)
Kesseler, K., 318 (132)
Khesin, M. J., 655 (20)
Killefer, O. H., 532 (41)
Killinger, J. E., 60 (21), 513 (41)
Kindscher, H., 318 (133)
Kindt, 318 (134)
King, H. H., 116 (48)
King, J., 690 (28)
King, P., 60 (50)
King, W. S., 533 (95)
Kingsford, T., 59 (1), 60 (29)
Kirchhoff, G. S. C., 403 (1)
Kirchhoff, M., 438 (6)
Kitchen, H., 241 (92), 317 (99)
Kjeldahl, M. J., 438 (21)
Kldiashwili, A. G., 318 (135, 136)
Kline, G. M., 323 (G, t)
Kluyver, A. J., 533 (83)
Kneen, E., 438 (3, 10, 13), 439 (35, 42, 47, 50, 59, 75, 78, 79), 440 (80, 81, 82, 92, 93, 122), 441 (131), 453 (11, 13), 532 (24, 25, 26, 36, 37), 566 (8)
Knehe, E., 314 (13)
Knoevenagel, E., 322 (A, l)
Knop, L., 320 (212 b)
Koenig, L., 318 (137)
Koester, H., 317 (102)
Koets, P., 244 (192, 193)
Kolachow, P. J., 531 (10)
Kolfenbach, J. J., 534 (108)
Kolthoff, J. M., 475 (16)
Kooi, M., 690 (34)
Kool, C. M. H., 85 (27)
Kopke, 316 (82)
Koszalka, W. A., 534 (114)
Kothe, H., 404 (44)
Kott, A., 405 (72)
Krajnc, B., 240 (14)
Kraemer, E. O., 318 (138)
Kraicinovic, M., 690 (64)
Kraus, A., 657 (95)
Krause, 372 (3)
Krause, C. B., 440 (112)
Krieger, J., 373 (43)
Krizkowsky, F. L. P., 405 (66), 655 (31)
Kroner, W., 404 (44)
Kuhn, R., 440 (86)
Kuhn, W., 318 (161), 404 (13, 14, 15)
Kulka, D., 440 (101)
Kummerow, F. A., 60 (13), 116 (48, 50)
Kunz, A., 320 (207)
Kunz, W. C., 655 (34, 37)
Kunze, W. G., 86 (59), 314 (17), 373 (54)
Kutzelnigg, A., 320 (244)
Kutzer, F., 373 (58)

L

Laidler, K. J., 258 (7, 9)
Landes, C. G., 512 (32, 40)
Landis, J. A., 567 (33)
Landis, Q., 178 (41), 438 (28), 566 (1)
Lane, J. H., 115 (3)
Langbein, G., 318 (139)
Lange, H., 322 (A, n)
Langford, C. T., 116 (22), 531 (17)
Langhans, A., 323 (G, o)
Langlois, D. P., 405 (67, 68, 77), 453 (20), 566 (16)
Langlykke, A. F., 242 (119), 438 (15), 440 (100), 441 (125)
Langwill, K. E., 567 (44)
Lansky, S., 690 (34)
Larmour, R. K., 567 (32)
Larmouth, G. W., 405 (82)
Larsen, D. H., 656 (65)
Lassman, M., 319 (195)
Lathrop, E. C., 532 (59), 534 (110, 111, 112)
Latker, S. N., 244 (226)
Lauffer, M. A., 243 (159)
Law, D. J., 318 (140)
Lazar, O., 438 (5)
Leavenworth, C. S., 689 (18)
Leberle, H., 440 (113)
Ledingham, G. A., 115 (14), 533 (93, 96, 97, 102)
Lee, H. N., 512 (1)
Lee, W. B., 626 (8)
Legg, D. A., 532 (49, 51)
Lehrman, L., 243 (172, 177, 178), 244 (182), 476 (57)

Leibowitz, J., 319 (196)
Lejeune, G., 85 (53)
Le Mense, E. H., 440 (100), 441 (125)
Lenders, A. W. H., 61 (57), 373 (34, 49)
Lengel, J. H., 533 (77)
Lenhard, W., 320 (205)
Lenze, F., 321 (261)
Lepow, I. H., 178 (46)
Leroy, 475 (2)
Leslie, J. O., 533 (99)
Leuck, G. J., 477 (99)
Levberg, 567 (40)
Levine, M., 241 (81), 318 (141), 342 (19), 403 (4), 690 (50)
Levy, H., 315 (57)
Lewis, K. H., 439 (49), 532 (24, 25)
Lieben, A., 318 (142)
Liebig, J., 318 (143), 342 (1)
Liebknecht, O., 405 (48, 57, 58)
Liechti, L., 318 (144)
Lienle, R. H., 588 (16)
Liggett, R. W., 239 (2), 404 (19, 21)
Lilienfeld, L., 318 (145, 146, 147)
Lilly, C. H., 533 (63)
Linder, W. V., 533 (65)
Lindsley, C. H., 153 (35)
Ling, A. R., 17 (4), 244 (187), 533 (66), 655 (10)
Link, K. P., 318 (148), 319 (166), 342 (17)
Lintner, C. J., 318 (149), 438 (22)
Little, J. E., 440 (108, 109)
Littmann, O., 317 (106 b, 106 c)
Lobenstein, M., 320 (210), 342 (22)
Lockhead, A. G., 533 (92)
Lockwood, L. B., 533 (89), 534 (123, 124, 125, 129)
Loeffel, W. J., 531 (11)
Loetscher, E. C., 477 (102)
Lohmann, K., 244 (219)
Lolkema, J., 85 (27), 318 (150, 151)
Lorand, E., 318 (152, 153)
Lottermoser, A., 476 (25, 26)
Lovell, C. L., 534 (104)
Lowe, D. V., 512 (7)
Lowe, G. M., 153 (29)
Lowy, K., 404 (46)
Lucht, O. C., 439 (39)
Ludecke, W., 532 (62)
Ludewig, S., 314 (14)
Ludwig, N. C., 656 (70)
Lüdtke, M., 690 (62)
Lundberg, B., 242 (99)
Lunden, R., 242 (110)
Lung, K. H., 241 (72, 73)

M

MacAllister, R. V., 405 (50), 689 (6)
McBain, J. W., 626 (5, 8)
Macbeth, A. K., 317 (118)
McBurney, J. D., 318 (157)
McClenahan, W. S., 438 (32)
McCloskey, C. M., 241 (83)
McCoy, E. F., 532 (52)
McCoy, R., 60 (41, 42, 43)
McCready, R. M., 177 (28), 240 (45), 241 (70), 244 (206), 476 (47), 690 (43)
MacDonald, J., 317 (120)
McDonald, J. W., 85 (22), 588 (14)
McDonald, J. Y., 241 (77)
McDonnell, C. C., 116 (30)
Macey, A., 244 (211), 438 (11), 441 (142)
Macfarlane, W. S., 626 (16)
Mach, G. F., 626 (18)
Machemer, H., 323 (G, j)
Mack, D. E., 318 (154)
McLean, D. A., 626 (15)
MacMasters, M. M., 17 (14, 21), 60 (16), 115 (9, 12), 116 (49), 655 (9, 11, 12)
MacMichael, R. F., 152 (7)
Macmillan, J. R., 318 (133)
McNeely, M., 116 (44)
MacNider, G. M., 152 (1)
Macormac, A. R., 588 (2)
McPherson, W. K., 531 (9)
McRary, W. L., 689 (4)
Maegher, W. R., 244 (227)
Märcker, M., 438 (9)
Mager, J., 689 (6)
Maggi, H., 476 (64), 477 (69)
Magnusson, B., 242 (123), 404 (7)
Maiden, A. M., 566 (21)
Maier, W., 86 (79)
Majmin, R., 244 (194)
Maksorov, B. V., 318 (155)

Malaprade, L., 242 (131), 318 (156), 343 (28)
Mallatt, R. C., 316 (87)
Maltby, W., 373 (28)
Mangels, C. E., 567 (32)
Maquenne, L., 240 (16), 476 (35), 566 (11)
Mark, H., 243 (158), 318 (161), 477 (85)
Markley, M. C., 567 (31)
Marshak, S., 534 (115)
Martelius, B., 242 (102)
Martens, P., 153 (24)
Martin, F., 321 (245)
Maskell, E. J., 244 (202)
Mason, M. J., 84 (8)
Massey, P. J., 512 (27)
Mast, W. C., 533 (75)
Mathiessen, C. H., 373 (43)
Mauersberger, H. R., 588 (1, 2, 3)
Maxwell, R. W., 85 (26), 477 (83)
May, O. E., 533 (67)
Mayer, A., 240 (11), 320 (208, 214), 477 (71)
Mayer, K., 441 (133)
Maynard, C. W., Jr., 322 (E, j), 343 (44)
Mecham, D. K., 441 (146), 453 (4), 566 (4)
Mehltretter, C. L., 656 (81)
Meisel, H., 242 (118), 373 (50), 405 (51, 76), 439 (58), 453 (5), 566 (17), 567 (46)
Meiss, P. E., 319 (174)
Mench, J. W., 318 (157)
Merrill, G. R., 588 (2)
Merrill, J. J., 61 (82), 373 (55)
Merritt, P. P., 567 (37)
Mertzweiller, J. K., 241 (86))
Meulen, J. H., van der, 318 (159)
Meusel, E., 85 (51)
Meyer, A., 17 (18), 177 (3)
Meyer, C. H., 373 (39)
Meyer, K. H., 17 (17, 19), 85 (37), 177 (1, 25), 178 (33), 240 (21, 22, 23, 35, 44), 241 (94), 242 (95, 120), 318 (160, 160 a, 161, 162), 323 (G, k), 439 (63, 64, 65, 66, 67), 440 (110), 476 (56), 477 (74), 690 (35, 49, 51)
Meyer, L. W. A., 316 (69)
Meyer-Delius, M., 241 (63), 316 (76)
Meyerhof, O., 244 (213)
Meyersohn, P., 319 (197)
Michael, A., 319 (163)
Michell, J. H., 243 (139, 140, 141), 322 (E, f), 323 (E, n, o), 343 (40, 42, 45)
Middleton, E. B., 319 (164)
Mienes, K., 322 (B, a), 323 (G, m)
Mikus, F. F., 244 (184), 476 (55)
Militzer, W., 440 (81, 82)
Millar, J. H., 315 (27)
Miller, A., 404 (26)
Miller, B. S., 440 (80), 532 (37)
Miller, J., 404 (43)
Miller, J. C., 116 (45)
Milner, M., 60 (17)
Milner, R. T., 534 (117), 689 (10)
Minto, W. L., 655 (18)
Mize, M. D., 567 (25), 689 (21)
Möller, F. A., 86 (73), 318 (151), 477 (92), 512 (35)
Moelvyn-Hughes, E. A., 404 (24), 438 (17, 18)
Moffatt, A. U., 61 (86)
Moffett, G. M., 60 (44)
Moissan, H., 319 (165)
Monroe, F. A., 405 (73)
Montgomery, E. M., 242 (115)
Monti, R. N., 405 (78)
Moore, E. E., 355 (2)
Moore, M. C., 86 (66)
Moore, S., 319 (166), 342 (17)
Morell, S. A., 534 (111, 112)
Morgan, W. L., 153 (40)
Morris, D. L., 689 (6)
Morris, G. H., 177 (7), 441 (145)
Morris, S. G., 373 (33)
Morris, V. H., 115 (11)
Morrison, G. A., 240 (48)
Moskowitz, M., 153 (28), 404 (11)
Mossgraber, E., 323 (G, l)
Mottier, D. M., 17 (16)
Moyer, W. W., 404 (12, 16, 29), 405 (68)
Mühlhauser, O., 319 (167)
Müller, F., 152 (10)
Müller, G. F., 152 (14)
Mullen, J. W., 17 (2), 86 (58), 153 (41), 177 (27), 258 (10), 319 (168, 169,

170, 178, 179, 180), 453 (9), 512 (13), 657 (92)
Muller, T. H., 61 (58)
Munson, L. S., 689 (1)
Murrav, T. F., 690 (56)
Murray, H. D., 476 (41)
Mustakas, G. C., 534 (109)
Mylius, F., 475 (4)
Myrbäck, K., 241 (85), 242 (96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 123), 403 (6), 404 (7), 438 (25), 440 (107), 476 (24)

N

Naegeli, C. W., 242 (128)
Nagel, C. M., 60 (19)
Nair, J. H., 690 (30)
Nanji, D. R., 244 (187), 655 (10)
Natta, G., 478 (112, 113)
Naylor, N. M., 440 (95, 96)
Neal, S. M., 153 (29), 588 (7)
Neish, A. C., 533 (94, 101, 102)
Nelson, G. E. N., 534 (125)
Nelson, J. M., 243 (171), 323 (F, c)
Nelson, M. L., 177 (10)
Neuberg, C., 532 (60, 61), 534 (130)
Neuberger, H. C., 314 (11)
Neukom, H., 477 (87)
Neumann, F., 241 (82)
Neumann, H. T., 86 (69)
Nevell, T. P., 690 (63)
Newkirk, W. B., 404 (11), 405 (84, 85, 86, 87)
Newton, J. M., 243 (146), 319 (171), 342 (18), 440 (95, 96)
Nichols, P. L., 657 (97, 98)
Nichols, P. L., Jr., 319 (172, 173, 174), 322 (B, b)
Nickerson, R. F., 177 (9)
Nicolson, J., 323 (E, p), 343 (49), 656 (85)
Nielsen, J. P., 689 (8)
Niemann, C., 689 (6)
Niemann, C. G., 318 (148)
Nieuwenhuis, K. L., 655 (25)
Nobel, A., 319 (175), 655 (35)
Noelting, G., 240 (44), 690 (51)
Nollau, E. H., 318 (157)
Nord, F. F., 533 (68)
Northrop, J. H., 243 (165), 323 (F, c), 534 (132)
Noznick, P. P., 567 (37)
Nussbaum, J., 314 (6)
Nusslein, J., 85 (24), 655 (26)
Nutall, N. H., 85 (56, 57), 477 (89), 588 (15)
Nycander, G., 242 (105)

O

Oeser, E., 321 (264)
Ohle, R. L., 438 (14)
Ohlson, E., 440 (87, 88)
Okada, H., 319 (176)
Olcott, H. S., 60 (23), 477 (75, 76, 77, 78, 79, 80)
Oldham, J. W. H., 323 (E, q), 343 (43)
Olson, W. J., 439 (36, 37)
O'Neill, C., 373 (19)
Onsdorff, T., 404 (43)
O'Reilley, P., 372 (11)
Ortenblad, B., 242 (103, 104, 106)
Osborne, T. B., 439 (60, 61)
Osgood, G. H., 512 (34)
Oshima, K., 440 (117)
Ost, H., 319 (177)
Othmer, D. F., 534 (113, 114, 115, 116)
Ott, Emil, 258 (2)
Owen, W. L., 532 (28)

P

Pacsu, E., 17 (2), 86 (58), 153 (41), 177 (27), 242 (125, 126), 258 (10), 319 (168, 169, 170, 178, 179, 180), 404 (34), 453 (9), 512 (13), 657 (92)
Padoa, M., 476 (22)
Paine, H. S., 116 (32, 35, 36, 38), 404 (40, 41)
Palit, C. C., 319 (181, 182), 342 (12, 13, 14, 15)
Pancirolli, F., 319 (183)
Parker, H. K., 567 (25), 689 (21)
Pascoe, E. D., 115 (11)
Pattilloch, D. K., 477 (104)
Paul, P. T., 404 (27)
Pauling, L., 258 (1)
Pauser, K., 85 (24), 655 (26)

Payen, A., 319 (184), 438 (7), 475 (12, 13)
Peat, S., 178 (31), 240 (39), 241 (69, 76, 84, 92), 244 (205, 209, 210, 211), 317 (99), 438 (11), 441 (142, 143, 144)
Pecker, A. G., 656 (48)
Pecker, E. A., 656 (48)
Pecker, J. S., 656 (48)
Peckham, G. T., Jr., 405 (80), 533 (79)
Peiser, E., 319 (185)
Pelouze, J., 319 (186, 187), 342 (2)
Peltier, G. L., 441 (131, 132), 532 (26)
Peltzer, A., 61 (61, 62, 63, 64, 65, 66, 68)
Peltzer, A., Jr., 61 (61, 62, 63, 64, 65)
Peniston, Q., 404 (31)
Penn, W. S., 319 (188)
Perboner, S., 657 (104)
Percival, J., 17 (13)
Percival, E. G. V., 239 (4)
Perges, N., 534 (129)
Perkin, W. H., 532 (39)
Perkins, F., 656 (50, 51)
Persoz, J., 438 (7)
Peters, A. F., 656 (49)
Petersen, B., 655 (27)
Peterson, W. H., 532 (48), 534 (133)
Petit, P., 319 (189)
Pettijohn, O. G., 533 (87, 88, 89)
Pfaehler, K., 242 (132), 318 (156), 343 (29)
Pfleger, R., 317 (106 c, 107, 108)
Phelps, J. K., 244 (180)
Philipp, H. J., 177 (10)
Phillips, N. C., 373 (59)
Phol, E., 512 (7)
Phol, J. J., 475 (11)
Pickering, S., 475 (8)
Pictet, A., 86 (87)
Pierce, F. T., 588 (12)
Pierson, G. G., 85 (35, 49), 323 (E, r), 343 (56), 477 (96), 655 (8), 657 (105)
Pigman, W. W., 241 (91), 319 (190)
Pinel, J. F., 373 (27)
Pinnow, J., 475 (14)
Plant, M. M. T., 240 (51), 317 (99)
Ploetz, T., 316 (79), 476 (34)
Pochin, H. D., 373 (17)
Pokorny, J., 323 (G, n)
Porst, E. G., 153 (28)
Porter, R. W., 478 (110)
Posternak, T., 243 (167), 319 (191)
Preece, I. A., 439 (41)
Pregl, F., 319 (192, 193)
Preuss, E., 115 (4)
Pringsheim, H., 319 (194, 195, 196, 197), 323 (G, o, p)
Pucher, G. W., 689 (18)
Pulfrey, A., 60 (51)
Purves, C. B., 243 (139, 140, 141, 142), 315 (42), 322 (E, e, f), 323 (E, n, o), 343 (39, 40, 42, 45)
Pygrof, 323 (E, s)

R

Rabinowitsch, B., 153 (31)
Radford, H. E., 656 (58)
Radley, J. A., 115 (5), 152 (13), 319 (198), 323 (G, g), 342 (5), 343 (59), 475 (10), 655 (33), 657 (94)
Raistrick, H., 534 (137, 138)
Rall, 404 (37)
Rampacek, C., 656 (73)
Rankin, D., 86 (68), 322 (B, c)
Rankin, E., 315 (50 a)
Rapp, W., 316 (78)
Rask, O. S., 152 (5), 153 (37), 244 (180), 689 (16)
Rassow, B., 320 (210), 342 (22)
Ratchford, W. P., 533 (74, 76)
Rawlins, A. L., 86 (96)
Rayburn, C. N., 534 (135)
Rayman, M. M., 532 (58)
Record, B. R., 241 (61)
Redfern, S., 438 (28), 439 (38, 74), 440 (84, 102)
Reed, D. L., 534 (109)
Reed, G. N., 152 (16), 355 (3)
Rees, D. E., 241 (83)
Rees, G. E., 319 (196)
Reeves, M. D., 534 (124)
Reeves, R. E., 178 (39)
Rehwald, F., 115 (2)
Reich, W. S., 319 (199, 200), 323 (G, r)
Reichardt, 318 (142)

Reichert, E. T., 17 (5)
Reilly, J., 323 (G, s)
Reinfurth, E., 532 (61)
Reinhart, F. W., 323 (G, t)
Reinsch, H., 319 (201)
Reintjes, H. R., 60 (51)
Reppe, 657 (96)
Reschke, C., 656 (44, 45)
Reychler, A., 319 (202), 320 (203)
Reyle, B., 322 (A, j)
Richardson, W. A., 85 (30), 243 (147), 241 (55), 317 (110), 323 (E, t), 343 (37), 690 (48)
Richee, W. R., 116 (35, 36)
Riess, A., 656 (86)
Rigamonti, R., 477 (109)
Ripperton, J. C., 243 (164)
Rist, C. E., 115 (21), 690 (65)
Ritchie, G. G., 321 (256)
Ritter, F., 320 (204)
Roach, J. R., 690 (60)
Roberts, C. F., 475 (5)
Roberts, R. H., 318 (148)
Roberts, W., 438 (23)
Rodebush, W. H., 258 (6)
Rodewald, H., 476 (27)
Roe, A. S., 317 (119)
Roe, E. T., 534 (129)
Röhm, R., 318 (132)
Rogge, R. H., 405 (64)
Roll, F., 656 (86)
Rose, D., 440 (105), 533 (95)
Rosenthal, L., 320 (205)
Rosted, C. O., 439 (46)
Roudier, A., 243 (145)
Rouvier, G., 476 (20)
Roux, E., 240 (16), 476 (35)
Rowland, B. W., 85 (20), 512 (3, 4)
Rudy, H., 322 (A, m)
Rumsey, L. A., 566 (14)
Rundle, R. E., 177 (14, 16, 18, 19, 21), 178 (35, 36, 37), 240 (28, 29, 46), 243 (148, 149, 150, 151, 152), 244 (184), 321 (A, a), 323 (G, u, v), 476 (37, 38, 39, 40, 42, 45, 49, 50, 55), 690 (40, 44)
Rutherford, J. K., 323 (E, g), 343 (43)

S

Saare, O., 115 (1), 153 (24)
Sadler, S. S., 655 (36)
Sadovy, I. E., 404 (23)
Sadtler, S. S., 320 (206, 207)
Saeman, J. F., 404 (25)
Sagrott, P. E., 240 (39)
Sair, L., 153 (45), 690 (24)
Sakurada, I., 320 (207 a)
Samec, M., 85 (17, 47, 50), 153 (39), 240 (10, 11, 12, 13), 242 (113, 114), 244 (188, 190), 319 (208, 209, 210, 211, 212 a, 213, 214), 342 (20, 21, 23), 404 (39), 477 (71, 72, 88), 512 (14), 689 (23)
Samsa, E. G., 177 (23)
Samuel, J. O., 657 (111)
Sandstedt, R. M., 17 (10, 11), 153 (42), 438 (3), 439 (35, 42, 59, 75, 78, 79), 441 (146), 453 (4, 11, 13), 532 (36), 566 (4, 8, 9, 23)
Sandvik, O., 153 (36)
Sapedina, P. M., 404 (10)
Sapirstein, L. A., 60 (23)
Sapozhnikov, A. V., 320 (215)
Saxl, I. J., 512 (23)
Savarè, B., 476 (22)
Savary, J., 440 (106)
Sawdon, W. A., 656 (52, 63, 64)
Scalf, E. E., 438 (12)
Scarborough, N. F., 567 (45)
Schaaf, E., 316 (79), 476 (34)
Schaeffer, G., 320 (216)
Schaer, E., 85 (52), 475 (17)
Scheffer, C., 533 (83)
Scheurer, F., 320 (217)
Scheurer, O., 320 (216)
Schieltz, N. C., 321 (260)
Schilling, R. H., 115 (17, 18)
Schink, N. F., 85 (21), 242 (117, 118), 373 (50), 403 (5), 405 (76), 439 (57, 58), 453 (5, 19), 477 (91), 512 (15, 19, 21), 566 (15, 17), 567 (49), 656 (68), 690 (42)
Schinle, R., 315 (26)
Schlechter, N., 534 (113, 114, 115, 116)
Schmerber, O., 320 (217)
Schmid, L., 320 (218, 219)

Schmidt, A., 322 (A, n)
Schmidt, E., 320 (220)
Schmidt, G., 244 (207)
Schmidt, O., 478 (114)
Schmidt-Nielson, S., 689 (22)
Schniepp, L. E., 534 (110, 112)
Schnurmann, R., 626 (12)
Schoch, T. J., 84 (2), 153 (38), 177 (17, 24, 26), 239 (7, 8), 240 (30, 32, 33), 244 (181, 183), 258 (8), 320 (221, 222), 355 (5), 453 (7), 476 (54, 59, 60, 61), 567 (38), 690 (34, 47)
Schoenbein, C. F., 476 (19)
Schoene, L., 440 (119), 453 (14, 15)
Schollenberger, J. H., 59 (4)
Scholten, W. A., 323 (G, w)
Schopmeyer, H. H., 61 (81), 84 (1, 12), 116 (52), 323 (E, u), 343 (58), 405 (82), 533 (80), 567 (48)
Schrader, R., 61 (60)
Schroeder, C. W., 690 (30)
Schroeder, W. C., 531 (2)
Schryver, S. B., 244 (185, 186)
Schubert, S., 317 (113, 114)
Schudel, G., 321 (262)
Schüler, H., 61 (53)
Schuetz, R. D., 404 (35)
Schützenberger, P., 320 (223)
Schultz, A., 441 (129)
Schultz, A. S., 567 (33)
Schulz, G. V., 318 (162), 320 (229 c)
Schwarz, E. W. K., 588 (1)
Schweitzer, O., 320 (234)
Schwimmer, S., 439 (70), 441 (147)
Seborg, R. M., 626 (14)
Seeborg, E. F., 690 (25)
Seiberlich, J. K. H., 343 (60)
Semeniuk, G., 60 (18, 19)
Senior, J. K., 534 (132)
Senkus, M., 534 (107)
Senti, F. R., 177 (22), 178 (47)
Serberlich, J., 657 (106)
Severson, G. M., 177 (20), 240 (24), 318 (131), 440 (120), 453 (8), 476 (36), 512 (16), 532 (24, 29, 30, 31, 45, 46), 655 (17), 690 (45, 53)
Shane, R. S., 320 (224)
Sheets, G. H., 513 (43)
Shen, T., 566 (22)
Shepherd, G., 531 (9)
Sheppard, F., 316 (58), 343 (27)
Sherman, H. C., 240 (19), 243 (166), 320 (208), 439 (62)
Sherman, J. M., 534 (134)
Sherman, R. T., 243 (176)
Sherwood, F. E., 657 (109)
Shewfelt, A. L., 115 (13, 20)
Shiver, F. H., 116 (28, 29)
Shoemaker, S. O., 405 (71)
Shorigina, N. N., 241 (74)
Shreve, R. N., 318 (154)
Shriner, R. L., 405 (75)
Shurbock, S., 61 (56)
Sichel, A., 86 (72)
Sichel, Ferdinand, A. G., 567 (51)
Sichert, K., 404 (17)
Siemens, W. von, 320 (225)
Silin, P. M., 404 (10, 36)
Sillen, L. G., 242 (98, 107, 108)
Silmann, S. H., 319 (196)
Simerl, L. E., 476 (46)
Simha, R., 243 (162)
Sineguf-Lavremko, A., 657 (108)
Singer, P. A., 373 (51)
Singh, B., 404 (33)
Sjostrom, O., 17 (22), 60 (43), 84 (7), 85 (31), 320 (226), 404 (42)
Skraup, Z. H., 320 (227)
Slattery, M. C., 689 (4)
Slotter, R. L., 115 (9), 116 (22)
Smirnov, E. A., 404 (38)
Smith, C. E., 405 (79)
Smith, F. A., 317 (109)
Smith, L. T., 319 (173), 322 (A, p), 533 (71, 72, 73, 74, 75), 657 (98)
Smith, W., 314 (18)
Smitt, P., 405 (53)
Snelling, W. O., 319 (196), 320 (228), 6 (39, 40)
Sohns, W. E., 441 (125)
Somogyi, M., 439 (48)
Sostegni, L., 243 (170)
Speiser, R., 243 (157)
Spengler, H., 655 (13)
Spilka, S., 656 (47)
Spoerl, J. M., 438 (13)

Sprague, G. F., 60 (9), 323 (G, x)
Stacy, R. H., 656 (73)
Staley, A. E., 61 (76), 405 (81)
Stamberg, O. E., 566 (7)
Stamm, A. J., 626 (14)
Stanier, R. Y., 533 (96, 97, 98)
Stark, W. H., 438 (12), 441 (123), 532 (33, 34)
Starr, D. F., 532 (50)
Staub, A., 440 (110, 111)
Staub, J., 84 (6)
Staud, C. J., 690 (56)
Staudinger, H., 178 (43), 241 (56, 57, 58), 316 (75), 318 (162), 320 (229 a, 230, 231, 232, 233, 234), 322 (A, o), 690 (36)
Stearn, A. E., 438 (19)
Steele, H. K., 690 (52)
Stefaniak, J. J., 534 (127)
Stein, H., 321 (235)
Stein, R. S., 178 (37), 243 (152), 476 (50)
Steinbrunn, G., 318 (161), 404 (14)
Steiner, E. T., 689 (12)
Stenlid, G., 242 (105)
Stern, E., 321 (236)
Stern, R., 566 (24)
Steurer, E., 240 (15)
Stevens, A. H., 405 (81)
Stickley, E. S., 314 (7), 322 (C, a)
Stiles, H. R., 532 (51)
Stocks, H. B., 475 (3)
Stolle, 316 (82)
Stratton, S. T., 60 (28)
Straub, A., 439 (65)
Strohmaier, A. J., 534 (104)
Stubbs, J. J., 534 (129)
Studer-Pecha, H., 242 (112, 122), 439 (53, 54)
Stutzke, R. W. G., 86 (78), 373 (30, 40)
Suida, W., 318 (144)
Sumner, J. B., 689 (5)
Sunberg, R. L., 241 (83)
Supf, F,. 86 (85)
Sutermeister, E., 513 (45)
Sutherland, E. W., 244 (217, 218)
Sutra, R., 321 (237), 477 (82)
Swain, R., 405 (61)
Swanson, C. O., 567 (26, 34)
Swanson, M. A., 178 (32), 244 (203), 404 (8), 439 (52), 690 (32)
Swayze, M. A., 656 (69)
Syniewski, W., 321 (238, 239, 240), 342 (8, 25), 477 (65)
Sypiaguin, A. S., 405 (71)
Szego, E., 59 (2)

T

Taft, P. B., 477 (103)
Takamatsu, Y., 316 (83)
Takamine, J., 440 (115, 116)
Talley, E. A., 322 (A, p)
Tamba, R., 321 (241)
Tamm, 241 (85)
Tanko, B., 244 (220)
Tanret, C., 240 (17, 18), 512 (12)
Tanret, G., 177 (26)
Tarr, L. W., 153 (23)
Tartaron, F. X., 656 (74, 75, 76, 78)
Taylor, G. T., 532 (22, 38)
Taylor, R. L., 61 (77)
Taylor, T. C., 243 (171, 172, 173, 174, 175, 176), 258, (3, 4), 321 (242), 355 (1, 7)
Tempus, F., 317 (115)
Termansen, J. B., 655 (19)
Teunissen, H. P., 404 (30)
Thaler, H., 60 (14)
Thomas, A. W., 626 (3)
Thomas, E. M., 244 (186)
Thomas, J. J., 86 (61, 62), 373 (52, 53)
Thomas, M. P., 321 (243)
Thompson, A., 404 (28)
Thompson, R. R., 241 (93), 439 (68, 69)
Thomson, T. R., 321 (244)
Thorsell, W., 242 (100, 103)
Thurber, F. H., 116 (33, 34, 35, 36, 37, 38, 41)
Tiers, L. P., 61 (85)
Tilden, E. B., 438 (31, 32)
Timmermans, J., 321 (245)
Tokuzo, N., 321 (246)
Tomecko, C. G., 321 (247)
Toth, J., 476 (21)
Traquair, J., 85 (23), 315 (46), 321 (248)
Traube, W., 321 (249)
Treadway, R. H., 322 (A, g, r)
Trogus, C., 317 (108)

Truax, D. E., 85 (56, 57), 477 (89), 588 (15), 626 (1)
Trubell, O. R., 240 (26), 244 (179, 189), 318 (131), 453 (10), 476 (48), 690 (53)
Tryller, H., 323 (F, d)
Tsuzuki, Y., 321 (250, 251, 252)

U

Underkofler, L. A., 440 (119, 120, 121), 453 (14, 15, 16, 18), 531 (21), 532 (30, 31, 35, 57, 58), 533 (69, 70), 534 (128, 131)
Unruh, C. C., 242 (130), 321 (253)
Upson, F. W., 315 (51)
Urbanski, T., 319 (196)

V

Valenta, E., 152 (11)
Vance, J. E., 689 (3)
Van Voorst, F. Th., 689 (20)
Vickery, H. B., 689 (18)
Vietti, W. V., 656 (54)
von Bergen, W., 588 (3)
Van der Meer, W. A., 567 (50)
van Deventer, C. M., 476 (18)
van Hasselt, J., 84 (4)
van Itallie, T. B., 177 (12)
Van Lanen, J. M., 440 (100), 441 (125), 531 (3)
Van Linge, R. A., 323 (G, y), 657 (101, 102)
Van Slyke, D. D., 438 (20)
Verity, J., 372 (12)
Vernon, C. C., 532 (34)
Victorow, C., 315 (39)
Vincent, G. P., 84 (11), 343 (61)
Viswanath, B., 440 (94)
Vogel, A., 475 (9)
von Asboth, A., 342 (7)
von Buzágh, A., 626 (11)
von Euler, H., 476 (24)
von Kaufman, W., 477 (67, 68)
von Krauss, E., 317 (128)
Von Loesecke, H. W., 534 (120)
von Przylecki, St. J., 244 (194)

W

Wachter, A., 657 (89, 90)
Wagner, T. B., 60 (30, 31)
Wagoner, J. A., 152 (16), 355 (3)
Waine, A. C., 317 (99)
Wakeman, L. K., 372 (6)
Waksman, S. A., 531 (16), 534 (121)
Walden, M. K., 241 (93), 439 (68, 69)
Waldsmidt-Leitz, E., 242 (114)
Walker, P. H., 689 (1)
Wallerstein, L., 441 (130), 453 (10)
Walmsley, R. A., 534 (105)
Walsh, J. F., 86 (96), 405 (54, 65)
Walter M., 655 (15)
Walton, R. P., 243 (175), 321 (242, 254), 342 (4), 438 (1)
Ward, G. E., 533 (87, 88, 89), 534 (123)
Watson, S. A., 689 (17)
Watson, W., 318 (133)
Weakley, F. B., 242 (115)
Webb, J. I., 239 (6), 317 (100)
Weber, F., 477 (87)
Weill, C. E., 440 (90, 91)
Weinig, A. J., 656 (72)
Weinmann, H., 689 (19)
Weiser, H. B., 626 (1, 9)
Weiss, E., 477 (101)
Weiss, W., 512 (7)
Welch, M. S., 244 (215)
Wells, P. A., 534 (129)
Wengraf, P., 323 (G, z)
Werkman, C. H., 531 (1), 534 (135)
Werner, E. A., 626 (26)
Werntz, J. H., 243 (174)
Wertheim, M., 240 (23), 323 (G, k), 690 (49)
West, C. J., 321 (255)
Westhoff, F., 319 (177)
Wetzstein, H. L., 531 (15)
Whalley, M. E., 405 (88)
Whetstone, J., 241 (84)
Whistler, R. L., 240 (38), 320 (222), 321 (257, 258, 259, 260), 476 (58), 657 (93), 689 (17)
Whitaker, M. C., 657 (99)
Whittenberger, R. T., 243 (157)
Whittier, E. O., 534 (134)
Wickerham, L. J., 533 (89)

Widmer, J. M., 60 (40)
Widmeyer, 404 (37)
Wiegel, E., 61 (53, 54), 116 (25), 240 (34, 36)
Wiggins, L. F., 322 (A, k)
Wijsman, H. P., Jr., 440 (85)
Wiley, H. W., 98 (7)
Wilkinson, M. D., 240 (51), 317 (99)
Will, W., 321 (261)
Williams, A. E., 534 (122)
Williams, C. B., 244 (183), 476 (54)
Williams, M. F., 656 (73)
Willkie, H. F., 531 (10)
Willstaedt, E., 440 (107)
Willstätter, R., 321 (262)
Wilson, G. F., 372 (11)
Wilson, P. W., 534 (136)
Wilson, R. E., 655 (1)
Wimmer, E. L., 532 (35)
Winfield, J. R., 321 (256)
Winzor, F. L., 317 (118)
Wisthoff, R. T., 441 (124)
Witnauer, L. P. 177 (22)
Witt, C., 320 (225)
Witt, P. R., Jr., 438 (14)
Wobser, R., 152 (10)
Wohlgemuth, J., 438 (24)
Woker, G., 476 (63), 477 (69, 70)
Wolff, E., 240 (22), 318 (162), 690 (35)
Wolff, I. A., 690 (65)
Wolff, J., 316 (68), 321 (263), 342 (9)
Wolffenstein, R., 321 (264)
Wolfrom, M. L., 241 (90), 319 (190), 404 (35, 45)
Wolfsohn, K., 323 (G, p)
Wolter, R., 323 (G, s)
Woodard, O., 116 (45)
Woolley, J., 373 (17)
Woolgar, M. D., 240 (52), 317 (101), 343 (38)
Worden, E. C., 321 (265, 266, 267)
Wright, D. E., 405 (74), 534 (119), 689 (9)
Wright, R. E., 116 (45)
Wrigley, A. N., 322 (B, b)
Wulkan, H., 372 (10), 373 (35)
Wurster, C., 321 (268), 322 (6)
Wynkoop, R., 532 (44)

Y

Yanovsky, E., 317 (96), 319 (173, 174), 322 (A, r, B, b), 657 (98)
Yelland, W. E. C., 588 (4)
Yoshikawa, K., 477 (108)
Young, G., 321 (269)
Young, G. T., 241 (66, 67, 71), 242 (124), 317 (98, 111), 320 (233)
Young, H. H., 86 (70)

Z

Zega, Z., 317 (129)
Zfasman, E. M., 244 (226)
Ziese, W., 321 (270)
Ziifle, H. M., 177 (10)
Zulkowski, K., 321 (271), 626 (24)
Zwikker, J. J. L., 85 (46)

Subject Index

A

Acetaldehyde, action of on starch, 74, 468, 471, 547
  production of, 526
Acetic acid, 94, 526
Acetone, 524
Acid, reactions of starch with, 215, 347, 375, 377
Acyl derivatives of starch, preparation of, 262
  properties of, 277
Adhesive action, theory of, 590
Adhesives, 589
  bag seam gums, 604
  box gums, 609
  binder in molded products, 598
  carton sealing gums, 609
  cold water soluble gums, 609, 616
  criteria for, 594
  defoamers for, 622
  envelope dextrins, 600
  envelope seal gums, 601
  envelope seam gums, 603
  for corrugated board, 596
  for waxed paper, 605
  gelatinized *in situ,* 595, 612
  gummed paper, 607
  laminating, 596, 608
  manufacture of, 613
  paper tape, 607
  plasticizers for, 602, 620
  preservatives for, 623
Adhesives, theoretical considerations of, 590
  tube winding gums, 606
  types of starch for, 595, 613
  use of emulsions in, 624
  veneer glues, 599
  water-resistant, 472, 600, 611
  wetting agents for, 605, 620
Adsorption of amylose, 169, 182
A-fraction of starch, 190, 192, 195, 677
A_1-fraction of starch, 194; *see also,* crystalline amylose
A_2-fraction of starch, 194
Alcoholic precipitation of amylose, 168, 190
Aldehydes, action of, on starch, 73, 466, 547
Alkali amylose, 164
Alkali lability, of dextrins, 345
  of starch fractions, 169, 682
  value, 348, 679
Alkali number, 679, 682
Alkali starch, 262, 265, 295
Alkaline fluidity of starch, 133
Alkyl derivatives of starch, preparation of, 295
  properties of, 298
Allyl starch, 294, 297, 652, 685, 687
Amidex, 368, 550
Amijel, 81, 487, 550
A-modification of starch, 159, 465
Amylases, 407
  action of on starch, 443
  activity of, 410
  animal, 430
  cereal, 423
  classification of, 409
  crystalline, 420
  dextrinizing, 413, 424, 449
  in flour, 537
  microbial, 428
  molecular weights of, 420
  nature of, 420
  pancreatic, 430
  production of, 431
  saccharifying, 416, 424, 450
  sources of, 431
  stability and inactivation of, 421, 446
α-Amylase, 413, 424
  action of in flour, 539
  action on starch, 208, 215, 444
  stabilizers and accelerators for, 421, 430, 446
β-Amylase, 424
  action of in flour, 537
  action on amylopectin, 206, 210, 212
  action on amylose, 184, 206
  action on oxidized starch, 337
  action on starch, 206, 215, 450
  production of, 432
Amyliq, 444
Amylocytase, 437
Amylodextrin, 158, 171, 414
Amylograph, 127, 145

Amylopectin, 167, 171
  methylation of, 181, 201, 210, 212
  molecular weights of, 188, 225
  structure of, 200, 211, 213, 230
  sub-fractionation of, 188, 193
Amylophosphatase, 436
Amylo process for fermentation, 522
Amylose, 167
  amorphous form of, 465
  corn crystalline, 184, 194, 458
  complexes of, 162, 172, 455, 463, 465
  films and fibers of, 163
  methylation of, 181, 201
  molecular weights of, 188, 225
  potato crystalline, 186
  tapioca crystalline, 186
  structure of, 184
  sub-fractionation of, 188, 193
  water solubility of, 182, 194
α-Amylose, 232
β-Amylose, 232
Amylose-Iodine complex, 162, 172, 457
Amylose-Fatty acid complex, 463
Analyses for starch, methods of, 659
Analysis for starch by acid hydrolysis, 659
  by diastatic hydrolysis, 661
  by iodine colorimetry, 662, 664
  by polarimetry, 663
Analysis of starches, 667
  for ash, 670
  for lipids, 671
  for moisture, 667
  for nitrogen, 670
  for protein, 670
Analytical methods, for alkali number, 679
  allyl starch, 685, 687
  carbonyl groups, 688
  carboxyl groups, 687
  carboxymethyl starch, 685
  ether groups, 684
  ester groups, 683
  ferricyanide number, 680
  hydroxyl groups, 686
  iodine affinity, 676
  intrinsic viscosity, 675
  linear polymer content, 678
  qualitative identifications, 681
  starch modifications, 672
  water solubility, 673
Antibiotics, 530
Arcy, 444, 579
Arrowroot, yield of starch from, 98
  starch, manufacture of, 97
Association in starch, 166, 223, 249
  through hydrogen bonding, 249
  through water, 250

B

Bacillus macerans, amylase activity of, 170, 414
Baking powder, function of starch in, 558
Barley starch, 16
Beater sizing, 484
Birefringence, of amylose films, 163
  of flow, 176
  of oxidized starches, 329, 337
  of starch granules, 5, 158
B-fraction, 192, 223
B-modification of starch, 160, 465; *see also,* Fig. 52
Borax, action of on starch products, 82, 470, 487, 597, 616
Brabender Amylograph, 127
Branching in starch structure, 200, 211, 213, 230
Brazilian arrowroot, 16
Breadmaking, role of starch in, 536
Brice photometer, 149
British gums, 348, 357
  manufacture of, 357
  use of in sizing, 581
Bromine, action of on starch, 73, 305, 310, 327
Brookfield Viscosimeter, 127
Butanol, 524
  fractionation of starch by, 190
Butadiene, 528
Butylene glycol, 528

C

Calendar sizing of paper, 491
Canary dextrins, 350, 362, 600
Carbon disulfide, action of on starch, 305
Carboxymethyl starches, 264, 276, 297, 685
  in detergents, 634
Cassava, *see* tapioca
Centrifugal separation of starch, 56
Chain length of starch molecules, 199, 330

Chemical properties of starch, 179
Chloral hydrate, action of on starch, 79
Chloramine T (Aktavin), oxidation of starch by, 342, 579
Chlorinated starch, 305
Chlorine, action of on starch, 72, 305, 327
Chloroacetylated starch, 276
Chlorosulfonic acid, action of on starch, 304
Chromate, action of on starch, 470
Citric acid, 529
Clay coatings for paper, 494
    brush application of, 495
    fluxing agents for, 500
    machine application of, 504
    modification of starch for, 498
    thixotropy of, 507
    use of starch in, 494
    viscosity and plasticity of, 501
    water resistant, 509
Coacervation of protein and starch, 233
Cold paste body, 123
Cold water paste products, 81, 84, 368, 487, 550, 642, 650
Cold water soluble starches, 75, 609, 673, 674
Color of starch, 63, 149
Composition of the starches, 179, 181, 191
Confections, use of starch and starch products in, 559
Consistometer, 127
Cori ester 234
Corn Industries Viscometer, 130
Corn grain, 29
    composition of, 31
    distribution of starch in, 33, 34
    varieties of, 34
    wet milling of, 37
Corn sirups, composition of, 380, 398
    clarification of, 394
    fermentability of, 541
    manufacture of, 398, 452
Corn starch; *see also,* starch
    bleaching of, 64
    centrifugal separation of, 56
    color of, 63, 149
    composition and components of, 183, 188, 191
    gelatinization temperature of, 145-147
    granules, 14
    manufacture of, 29
    mobile, 67, 558
    modification of, 63
    odorless, 66
Crackers and biscuits, use of starch in, 548
Cross-linking reactions, 466, 470
Crystalline amyloses, 163, 184, 186, 194, 228, 458, 464, 678
Crystalline forms of starch, 159
Crystallites, 9, 158
Crystal structure of starch, 9, 158
Cycloamyloses, 170; *see also,* Schardinger dextrins.
Cyclohexanol, action of on starch, 82, 190

D

Degomma, 579
Derivatives of starch, 259
Dextrinic acids, 216
Dextrins, alkaline gums, 366
    British gums, 348, 357
    by acid hydrolysis of starch, 215, 361, 367
    by enzymic action on starch, 369, 413, 449
Dextrins, canary, 350, 362, 600
    classification of, 357
    gelatinization of, 13, 24
    limit, 210, 212, 369, 414
    manufacture of, 357
    nitric acid esters of, 352
    plasticization of, 361, 372, 580, 602, 618, 620, 625
    properties of, 345
    Schardinger, 170, 414
    structure of, 354
    use of, in adhesives, 600
        in paper sizes, 494, 504
        in textile sizes, 580
    white, 345, 362
Dextrose, 375, 380, 402; *see also,* glucose
    anhydrous, 402
    content of sirups, 380
    crystallization of, 402
    in breadmaking, 541
    interconversion of to levulose, 403
    production of, 401
β-Dextrose, 403
Diastafor, 579

Diastases, 407; *see also,* amylases
Diastatic power of dough, 538
Diazomethane, methylation of starch by, 294
Dichroism of starch sols during flow, 172, 228, 459
  of the amylose-iodine complex, 163, 458
Dicyandiamide, action of on starch, 80, 625
Diglucose borate, 470
Dimethylolurea, action of on starch, 74, 80
Dioxins from starch, 334
Disaggregation of starch, 216
Disorganized starch, 159, 254
Dudley pipette, measurement of paste viscosity by, 122

E

Electrolysis, oxidation of starch in, 337
Electrolytes, effect of on sedimentation rate of starch, 54, 92, 107
Electrophoresis of starch pastes, 181
End group assay, 198, 219, 221, 327, 330
Enzyme accelerators and stabilizers, 421, 430
Enzymes, activity of, 410; *see also,* : nylases
  amyloclastic, 416
  amylophosphatase, 436
  dextrinogenic, 208, 413, 449; *see also,* α-amylase
  α-glucosidases, 209, 418, 428, 437, 450
  limit-dextrinase, 416, 429, 437
  liquefying, 412, 444
  maltase, *see,* α-glucosidases
  papain, 542
  phosphorylase, 234, 437
  phosphatase, 239
  Q-enzyme, 237
  saccharogenic, 416, 450; *see also* β-amylase and α-glucosidases
Epichlorohydrin, action of on starch, 75, 294, 297, 470
Erythronic acid, 219, 331
Erythrose, 219, 330
Ethanol, 474, 519, 525
Ethylene oxide, action of on starch, 294, 297
Explosives, use of starch and its derivatives in, 635
Exsize, 579

F

Fatty acid esters of starch, 264, 276
Fatty acids, action of on starch, 75, 190, 232, 463
  complex formation of with amylose, 464
  in starch, 231
Feculose, 74, 263
Fermentability of sirups, 541
Fermentation chemicals from starch: acetic acid, 94, 526; acetone, 524; butanol, 524; butylene glycol, 528; citric acid, 529; ethanol, 519; gluconic acid, 529; glycerol, 526; itaconic acid, 529; lactic acid, 527; penicillin, 530; streptomycin, 530; tyrothricin, 530
  economics of manufacture, 515
  uses for, 518
Ferricyanide number, 680
Flexibility of amylose chains, 176
Flours, use of in adhesives, 84, 598
  use of in breadstuffs, 536
Fluidity of starch, 133
Formaldehyde, action of on starch, 73, 466, 486
  from starch by periodate oxidation, 221, 330
Formic acid, action of on starch, 275
  from starch by acid treatment, 378, 387
    by periodic oxidation, 220, 330
Formyl derivatives of starch, 263, 275
Fractionating agents for starch, 190
Fractionation of starch, 167, 181, 190
  by alcoholic precipitation, 168, 190
  by electrophoresis, 181
  by hot water extraction, 167, 182
  by nitroparaffins, 190
  by thymol, 190
  by use of adsorbants, 182
Fructose-6-phosphate, 238
Fuchs gel penetrometer, 138
Fuel, alcohol from starch for, 518
Fungal amylases, 428
  use of in fermentation, 522

G

Gelatinization of starch, 10, 127, 143, 165
    defatted, 253
    double range of, 144
    H-bond changes during, 250
    in situ, 595
Gelatinization temperature of starch, 143
Gel properties of starch, 134
    changes in with acid modification, 561
        with oxidation, 337, 561
Gel strength of starch, 134
        decrease in with oxidation, 337
        measurement of, 134
Gentiobiose, 378, 386
Glucose, 199, 380
    anhydrous, 402
    crystallization of, 402
    manufacture of, 401; alpha isomer, 402; beta isomer, 403
    production of from starch by α-glucosidase, 209, 400, 418
    synthesis of by phosphorylation reactions, 234
Glucose-1-phosphate, 234
Glucose-6-phosphate, 238
6-(α-Glucosyl)-glucose, 209, 386
Glucuronic acid, 327
Gluten, from corn, 50, 56
    overflow water, 56
    modification of starch with, 84
    wheat, 101, 536
Glycerol, 474, 526
Glycogen, 157, 171, 175
    methylation of, 200
    reaction of with iodine, 460
    structure of, 200
Glyoxal, 219, 330
Granules, compound, 4
    growth of, 7
    organization of, 7
    physical properties of, 158
    shape of, 4; *see also,* photomicrographs in Chapter I
    size of, 3
    striations on, 5
    the hilum of, 5
    x-ray patterns of, 159

H

Halogen derivatives of starch, 305
Halogens, oxidation of starch by, 309, 327, 364
Helical coil configuration, 160, 170, 185, 457
Hemiacetal linkages, 216
Heterogeneity, of amylopectin, 193
    of amylose, 185, 193
Hexahydro-trimethoxyfuro-dioxins, 334
Hexalin; *see,* cyclohexanol
Hexosans, 83
Hilum, 5
Höppler Viscosimeter, 125
Hot paste viscosity, 119, 122
Hot water extraction of starch, 167, 183
Hydrogen bonding, 217, 249
    changes in, during gelatinization, 250
        during dextrinization, 349
    water in starch held by, 68, 249
Hydrogenolysis of starch, 473
Hydrol, 378
    composition of, 386
Hydrolysis of starch by acids, 375
    commercial methods for, 398
    effect of molybdenum on, 398
    in fermentation mashes, 522, 527
    reactions involved in the, 377
    secondary catalysts for, 397
Hydroxy ethers of starch, 294, 297, 684
Hydroxymethyl furfural, 378, 388
Hypochlorite oxidation of starch, 339

I

Impermex, 642
Intrinsic viscosity, 188, 226, 675
Iodides, action of on starch pastes, 619, 625
Iodine, coloration of starch by, 195, 455, 622, 664, 678
    reaction with starch, 170, 195, 221, 455
    titration, potentiometric, 171, 195, 459, 676
Ion exchangers, 396
    use of in sugar clarification, 397
Isopropyl alcohol, 474

K

Ketene, acetylation of starch by, 273
Kinematic viscosity, 255
Kinetics, of acid hydrolysis of starch, 379
    of amylase action, 410, 417
Koji, 434

L

Lactic acid, 527
    in corn steepwater, 40
Laminated structure for starch molecules, 200
Lecithin, action of on starch, 82
Levulinic acid, 378, 387
Levulose, production of from starch, 403
Light scattering experiments on starch, 177
Limit dextrins, 210, 212, 414
    hydrolysis of, 210, 212
    methylation and structure of, 210, 212
Liquefaction of starch, 412, 444
    in desizing, 449
    in cereal mashes for fermentation, 416, 448
Liquefons, 413

M

MacMichael viscosimeter, 124, 502
Macromolecules of starch, 223
Maize starch, *see* corn starch
Malting, 432
Maltose, 199
    produced by acid hydrolysis, 380
        amylase action, 400, 416
Maltotetrose, 380
Maltotriose, 380
Malts, 423, 432
    amylases in, 423
Manihot roots, composition of, 87, 89
    processing of, 87
Manioc, *see* tapioca
Maranta starch, *see* arrowroot starch
Melamine-formaldehyde condensation with starch, 472, 509
Merco centrifuges for separation of starch, 56
Methanol, 474
Methylation, exhaustive, 198, 201
    of amylopectin, 181, 201, 210, 212
        acid made dextrins, 215, 216
        amylose, 181, 201
        glycogen, 200
        limit dextrins, 210, 212
        starch, 198, 295
        synthetic starch, 236, 237
Methylglucoses: dimethyl, 200, 201; 2,3,6-trimethyl, 198; 2,3,4,6-tetramethyl, 199
    separation of, 203
Micellar organization by hydrogen bonding, 252
Microscopy of starch, 14
Milling, *see* chapters on manufacture of starch, 29-115
Milo maize starch, manufacture of, 113
    properties of, 114
Mobility, 67, 151
Modified starches, 63
    evaluation of, 117
Modification of starch with gluten, 84
Mogul, 84, 650
Moisture, hydrogen bonded, 68, 250
Moisture in starch, 68, 152, 250
    determination of, 667
Molecular weights, 175-7, 188, 193, 212
Molybdenum, action of in starch hydrolysis, 398
Multiple amylose theory, 167

N

Nitric acid esters, of dextrins, 352
    of starch, 299
    preparation of, 301
    properties of, 302
Nitric acid, oxidation of starch by, 311
Nitrogenous residues in starch, 233
Nitroparaffins as fractionating agents, 190

O

Optical rotation of starch, 173, 663
Osmotic pressure, 177, 224
Oxalic acid, 219, 326, 331
Oxidation reactions in starch structure studies, 218, 331
Oxidized starches, 307, 325
    dextrinization of, 364
    manufacture of, 339
    properties of, 337

P

Papain, action of on flour, 542
    effect of on activating barley amylase, 538
Paper, coating of, 494
    use of starch in manufacture of, 338, 483
Pasting characteristics of starches, 127
Penicillin, 530
Pentasol, as a fractionating agent, 192
Periodic acid, oxidation of starch by, 218, 312, 328
Peroxides, oxidation of starch by, 313, 326
Phosphoric acid esters of starch, 303
Phosphorus, in starch, 182, 230, 470
    rôle in synthesis of starch, 239
Phosphorus oxychloride, action of on starch, 304
Phosphorus pentachloride, action of on starch, 305
Phosphorylase, 234, 437
Phosphorylation, of amylose, 304
    of glucose, 238
    of starch, 234, 304
Physical modifications of starch, *see* modified starches
Physical properties of starch, 157
Plasticity in starch pastes, 125, 139
Plasticizers, for adhesives, 80, 602, 620
    dextrins, 361, 602
    starch derivatives, 287, 298
    starch sizes, 80, 576
    use of glucose for, 621
Plastics, use of starch or derived products in, 519, 527, 598, 652
Polidase, 579
Polyoxyethylene stearate, action of on starch, 80, 548
Polyzime, 579
Potatoes, composition of, 89, 105
    yield of starch from, 106
Potato starch, components and composition of, 181, 196
    dextrinization of, 104
    granules, 15
    manufacture of, 104
    phosphate in, 182, 230
Potentiometric titration with iodine, 171, 195, 459, 676
Printing pastes, use of starch in, 585
Propylene glycol, 474
Protective colloid effect of starch, 147, 497
Protein in starch, 57, 233, 670
Pyroantimonate, action of on starch, 80, 470

Q

Q-enzyme, 237, 437

R

Rapidase, 579
Residual dextrin, *see* limit dextrin
Resins from starch, 519
    acrylate, 527
    water-insoluble, 471
    water-soluble, 471
Resonance interaction of amylose and iodine, 462, 463
Retrogradation of amylose, 167, 468
    of starch, by dehydration, 250
        effect of aldehydes on, 468, 547
        effect on iodine coloration, 468
        from V configuration, 160
        in fermentation mashes, 520
        in solid state, 167
        in solution, 166
        rôle of in staling of bread, 167, 545
Rice starch, 16
Rigidity of starch pastes, 138
Rhozyme, 444
Rubber, synthetic, starch as a raw material for, 516

S

Saare gel strength method, 135
Saccharification of starch by enzymes, 450
Sago starch, 15
    manufacture of, 114
Salad dressings, use of starch in, 555
Salicylates, action of on starch, 79
Salicylic acid, action of on adhesives, 625
Salts, effect of, on amylases, 421, 430, 446, 447
    on crystallization of dextrose, 385, 397
    on hydrolysis of starch by acid, 387, 392
    on sedimentation of starch, 54, 92, 107
    on starch pastes, 78, 619, 622, 625
Salts, per-, oxidation of starch by, 78, 341
Schardinger dextrins, (cycloamyloses), 170, 414

Scott test for paste viscosity, 119
Sedimentation of starch, 50, 92, 107
  rate studies, 175
Shortness in starch pastes, 141
Silica in starch, 233
Size of starch granules, 3, 14
Sorbitol, 473
Sorghum starch, 16, 112
Specific rotation, 173, 663
Spectrophotometry, analysis of starch by, 195, 660, 662, 664, 678
Staling of bread, 544
Stannates, action of on starch, 490
Starch acetates, 267
  preparation of, 267
  properties of, 277
Starch, acid hydrolysis of, 375
  allyl ethers, 294, 297, 685, 687
  p-aminobenzyl ether, 295
  benzoate 262, 277
  benzyl ethers, 294
  buffering action of, 390
  butyl ethers, 294
  butyrate, 276
  carboxymethyl, 264, 276, 297, 685
  chloro derivatives of, 306
  cinnamate, 266, 277
  cold water pasting, 81, 84, 368, 487, 550, 642, 650
  color of, 63, 149
  composition and structure of, 179
  derivatives of, 259
  dusting, 67, 548
  enzymic conversion of, 205, 369, 400, 410, 437, 443, 499, 507, 520, 579, 615, 661
  ethyl ethers, 298
  floury, 4, 14, 34
  for enzyme conversion, 446
  fractions, 169, 181, 188
    alcoholic precipitation of, 168, 190
    as anticoagulants for blood, 633
    as beater sizes, 490
    as clay coating adhesives, 498
    cotton adsorption of, 169, 182
    hot water extraction of, 167, 182
    in fermentation, 523
    iodine coloration of, 195, 662, 664, 678
    iodine titration of, 171, 195, 459, 676
    molecular weights of, 188, 225
    properties of, 169, 188
  glycollate, 264, 276, 297, 685
  high viscosity modifications of, 71, 340
  horny, 4, 14, 34
  hydroxy ethers of, 294, 297
  in adhesives, use of, 595
  in foods, use of, 535
  in paper making, use of, 338, 483
  in textile manufacture, use of, 338, 569
  laurate, 264, 276
  lauryl ethers, 294, 297
  linoleate, 264
  low viscosity modifications, 76, 338
  methyl ethers, 295
  mobile, 67
  mobility of, 151
  modified, 63, 433
  molding, 68, 561
  monoformate, 263, 275
  nitrates, 299
  non carbohydrate constituents of, 228
  optical properties of, 158, 173, 663
  oxidized, 307, 325, 336
  palmitate, 264, 276
  phenol-formaldehyde condensations with, 471
  proprionate, 276
  propyl ethers, 297
  reducing power of, 188, 221, 680
  sols as protective colloids, 147, 497
  sources of, 3
  sizes for: clay coating paper, 494; paper at the calenders, 491; paper pulp at the beaters, 484; textile warps, 569; textiles, finishing of, 582; tub sizing paper, 493
  stearate, 264, 276
  tables, 53
  thermophile free, for canners, 65, 73
  thin-boiling, 77, 581
  undecylate, 264
  V configuration of, 160, 162, 185, 457 457

urea-formaldehyde condensations with, 74, 472, 509
xanthate, 305
Starchates, metal, 633, 651
Starch-iodine complex, 170, 195, 455
spectrophotometry of, 195, 662, 664, 678
titration for the determination of, 171, 195, 676
Starch-sugars, clarification of solutions of, 394
production of, 398
Starches: arrowroot, 16; barley, 16; corn, 14; potato, 15; rice, 16; sago, 15; sweet potato, 16; synthetic, 169, 234; tapioca, 15; waxy maize, 17; waxy sorghum, 16; wheat, 15
description of individual, 14
photomicrographs of, 17-25
Steeping of corn, 37
agents for, 39
countercurrent systems, 43
single phase system, 42
three phase system, 46
wheat, 100, 104
Steepwater, 43
evaporation of, 58
Stormer viscosimeter, measurement of cold paste body by, 123
Striations, 5
Structure, chemical, 179, 196, 216, 331
Sucrose, biochemical relation to starch, 239
Sulfonated oils, as plasticizers, 577, 580, 622
Sulfuric acid esters of starch, 303
Sweet potatoes, yield of starch from, 108, 112
Sweet potato starch, 16
manufacture of, 108
Swelling of granules, 10, 165
of oxidized starches, 337
Syneresis in starch gels, 142
Synthesis of starch, 234
Synthetic starches, 169, 234
methylation of, 236, 237

T

Tabling of starch, 53, 106, 109, 113
Tapinol, 81
Tapioca starch, 15
components and composition of, 181, 196
manufacture of, 87
Tarr-Baker jelly tester for starch gel strength, 134
Textile manufacture, use of starch in, 338, 449, 569
Thin-boiling starch, 76
Thiocyanates, action of on starch, 79, 83, 619, 625
Thixotropy, in clay coatings, 507
in printing pastes, 586
in starch pastes, 141, 254, 613
Thymol as a fractionating agent, 190
p-Toluenesulfonchloramide, action of on starch, 342, 579
Tosylated starch, 277
Trichites, 9
Tub sizing of paper, 493
Tufgel, 81

U

Ultracentrifugal measurements on starch, 175, 228
Unit cell, parameters of the crystal, 160
Urea, action of on starch, 80, 83, 608, 618
Urea-formaldehyde, condensations of with starch, 74, 472, 509
with starch-gums, 600, 611
Use of starch in, adhesives, 589,
aluminum refining, 645
baking powder, 558
beater sizing, 484
canned foods, 564
cement, setting of, 644
ceramics, 627
clay coatings, 494
confections, 559
cosmetics, 631
crackers, 548
detergents, 633
emulsions, 634
explosives, 635
fermentation industries, 515
flotation of ores, 646
foods, 535
foundry operations, 650
laundry sizes, 636

- meat products, 564
- mining and metallurgy, 639
- oil well drilling muds, 639
- paper manufacture, 483
- pharmaceuticals, 631
- pie baking, 563
- plastics, 598, 652
- printing pastes, 585
- puddings, 562
- salad dressings, 555
- textile manufacture, 569
- tub sizing, 493
- warp sizing, 569
- water treatment, 654

V

- Vanzyme, 444
- V configuration, 160, 162, 185, 465
- Viscosimeters, 119
- Viscosity, changes in during chlorination of starch, 340
    - dextrinization, 345
  - intrinsic, 226, 675
  - measurement of in pastes, 119
  - of starch derivatives, 278, 282
  - of starch fractions, 188
  - of starch pastes, 117

W

- Warp sizing, 569
  - of continuous filament yarn, 579
  - of spun yarn, 581
- Water retention of drilling muds, 640, 641
  - of gelatinized starch, 142
- Waxy maize starch, 17, 112
    - composition of, 189
  - sorghum starch, 16, 112
    - composition of, 189
- Wetting agents, action of with adhesives, 605, 620
- Wheat starch, granules, 15
  - manufacture of, 99
    - by Alsatian method, 100
    - Halle process, 101
    - Martin process, 101
    - newer methods, 102
  - in flour, 101, 536
  - phosphorus in, 231

X

- X-ray diffraction patterns: A, B, C, and V types, 159
  - of alkali amylose fibers, 164
  - of amylose films, 163
  - of corn crystalline amylose, 185
  - of purified corn amylopectin, 187

Y

- Yuca, *see* manihot and tapioca

hypochlorite. After the reaction is allowed to proceed for a specified period of time, the starch milk is screened, washed on continuous filters, resuspended, the pH of the slurry adjusted, and the starch filtered and dried. In this manner a sago starch product pure white in color and substantially free of impurities is obtained. The degree of modification of the finished product is controlled by the extent of the preliminary chlorine treatment.

The other large class of chemical compounds used in the preparation of oxidized starch is the per compounds; *e.g.*, sodium perborate, ammonium persulfate, alkaline peroxides, and alkaline permanganate. The usual practice with perborate and persulfate is to mix about 1% of the reagent with the air-dried starch. A slight physical modification is then brought about when the mixture is suspended in water and cooked to a paste. In other cases the starch paste is prepared, the oxidizing agent added, and cooking continued until modification is complete. In both methods, with either perborates or persulfates, only moderately modified starches are obtained. Persulfates will also yield modified starches by the wet process (54). The persulfate is added to the starch slurry, the correct amount of acetic or hydrochloric acid added, and the reaction is allowed to proceed at room temperature. The oxidized starch is recovered by filtration, washed thoroughly, and then dried.

As with the perborates and persulfates, alkaline peroxides, especially barium peroxide, can be mixed with the dry starch (55, 56). In the dry state or in aqueous suspensions at room temperatures there is little or no modification. Upon heating the aqueous suspension, pronounced modification occurs, the degree of modification depending on the quantity of peroxide added and the length of time the aqueous suspension is heated. The wet process is also applicable when peroxides are used as the oxidizing agent (57). In this method calcium peroxide is added to the starch slurry, which is heated to 52°C. (126°F.) and maintained at that temperature for about 24 hours. The oxidation mixture, which has a pH of about 11, is neutralized to pH 7.0 with hydrochloric acid, the water removed by filtration, resuspended in water, filtered and washed to remove the soluble salts, and then dried. Starches prepared in such a manner are thin boiling but thick setting.

The treatment of moist starch with from 0.05 to 0.15% hydrogen peroxide at 100-110°F., followed by drying in a Buell drier, yields starch free from thermophiles (58). No modification of the starch is produced.

Oxidation of starch by alkaline permanganate decreases the viscosity and increases the transparency of the pastes prepared therefrom. The modification can be continued until red-staining iodine reaction products are produced. Most "permanganate starches" have a yellowish tinge due to the retention of small traces of manganese compounds. Treatment with such agents as sulfur dioxide, sodium hydrosulfite, or oxalic acid destroys the residual color.

Sodium *p*-toluenesulfonchloramide, commonly known as chloramine-T or Activin is reported to be widely used for the commercial solubilization of starch in Europe. The chemical is said to solubilize the starch without any formation of dextrins or sugars (59). Oxidants for starch listed in recent patents are, sodium chlorite (60, 61) and hexavalent chromium salts (62).

The production of such a wide variety of oxidized starches to meet the specific requirements of the trade demonstrates the extent to which the art of starch oxidation has progressed. Practically nothing is known of the chemical changes which the starch molecule, and probably the granule, undergoes in these treatments. The polyfunctional nature of the starch molecule and the complex arrangement of such molecules into the highly organized starch granules make the problem extremely difficult. It is thus evident that the elucidation of the chemical and physical phenomena involved in the art of starch oxidation must lie in the results of future research investigations.

BIBLIOGRAPHY

1. Liebig, J., *Ann.*, **15**, 541 (1829).
2. Pelouze, J., *Compt. rend.*, **7**, 713 (1838).
3. Habermann, J., *Ann. chem. Pharm.*, **172**, 11 (1874).
4. Walton, R. P., A Comprehensive Survey of Starch Chemistry, The Chemical Catalog Company, New York (1928).
5. Radley, J. A., *Mfg. Chemist.*, **13**, 101, 158, 201 (1942).
6. Wurster, C., *Ber.*, **22**, 145R (1889).
7. von Asboth, A., *Chemiker-Ztg.*, **16**, 1517, 1560 (1892).
8. Syniewski, W., *Ber.*, **31**, 1791 (1898).
9. Fernbach, A., and Wolff, J. *Intern. Cong. Appl. Chem., 7th, London*, **6 b**, 124 (1909).
10. Durieux, O., *Bull. soc. chim. belg.*, **27**, 90 (1913).
11. Dhar, N. R., *J. Phys. Chem.*, **28**, 943 (1924).
12. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.*, **29**, 799 (1925).
13. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.*, **30**, 939 (1926).
14. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.*, **34**, 711 (1930).
15. Palit, C. C., and Dhar, N. R., *J. Phys. Chem.*, **32**, 1263 (1928).
16. Brown, W. R., *J. Biol. Chem.*, **113**, 417 (1936).
17. Moore, S., and Link, K. P., *J. Biol. Chem.*, **133**, 293 (1940).
18. Newton, J. M., Doctoral dissertation, Iowa State College, Ames, Iowa (1941).
19. Levine, M., Foster, J. F., and Hixon, R. M., *J. Am. Chem. Soc.*, **64**, 2331 (1942).
20. Samec, M., *Kolloid-Beihefte*, **28**, 155 (1929).
21. Samec, M., *Kolloid-Z.*, **64**, 321 (1933).
22. Rassow, B., and Lobenstein, M., *Kolloid-Beihefte*, **33**, 179 (1931).
23. Samec, M., and Blinc, M., *Kolloid-Beihefte*, **38**, 48 (1933).
24. Felton, G., Farley, F. F., and Hixon, R. M., *Cereal Chem.*, **15**, 678 (1938).
25. Syniewski, W., *Ann.*, **441**, 277 (1925).
26. Farley, F. F., and Hixon, R. M., *Ind. Eng. Chem.*, **34**, 677 (1942).

27. Everett, M. R., and Sheppard, F., Oxidation of Carbohydrates in Acid Solution, University of Oklahoma Medical School, Oklahoma City, Oklahoma (1936).
28. Malaprade, L., *Bull. Soc. chim.*, **43**, 683 (1928).
29. Karrer, P., and Pfaehler, K., *Helv. Chim. Acta,* **17**, 766 (1934).
30. Herissey, H., Fleury, P., and Joly, M., *J. pharm. chim.,* **20**, 149 (1934). See also, Halsall, T. G., Hirst, E. L., and Jones, J. K. N., *J. Chem. Soc.,* 1427 (**1947**).
31. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **58**, 378 (1936).
32. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **59**, 994 (1937).
33. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **59**, 2049 (1937).
34. Caldwell, C. G., and Hixon, R. M., *J. Biol. Chem.,* **123**, 595 (1938).
35. Jackson, E. L., and Hudson, C. S., *J. Am. Chem. Soc.,* **60**, 989 (1938).
36. Armstrong, E. F., and Armstrong, K. F., The Carbohydrates, Longmans, Green, New York (1934).
37. Richardson, W. A., Higginbotham, R. S., and Farrow, F. D., *J. Textile Inst.,* **27**, 131T (1936).
38. Haworth, W. N., Hirst, E. L., and Woolgar, M. D., *J. Chem. Soc.,* 177 (**1935**).
39. Grangaard, D. H., Gladding, E. K., and Purves, C. B., *Paper Trade J.,* **115**, 41 (1942). Heidt, L. J., Gladding, E. K., and Purves, C. B., *Paper Trade J.,* **121**, 81 (1945).
40. Grangaard, D. H., Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.,* **61**, 1290 (1939).
41. Baird, D. K., Haworth, W. N., and Hirst, E. L., *J. Chem. Soc.,* 1201 (1935).
42. Michell, J. H., and Purves, C. B., *J. Am. Chem. Soc.,* **64**, 585 (1942).
43. Oldham, J. W. H., and Rutherford, J. K., *J. Am. Chem. Soc.,* **54**, 366 (1932).
44. Hockett, R. C., and Maynard, C. W., Jr., *J. Am. Chem. Soc.,* **61**, 2111 (1939).
45. Michell, J. H., and Purves, C, B.. *J. Am. Chem. Soc.,* **64**, 589 (1942).
46. Hibbert, H., and Hill, H. S., *J. Am. Chem. Soc.,* **45**, 734 (1923).
47. Baker, W., and Field, F. R., *J. Chem., Soc.,* 86 (**1932**).
48. British Patent 534,503 (1941).
49. Nicolson, J., U. S. Patent 2,221,552 (1939).
50. Bryant, A. P., U. S. Patent 1,937,543 (1931).
51. Kerr, R. W., U. S. Patent 2,108,862 (1935).
52. Bergquist, C., U. S. Patent 1,851,749 (1927); U. S. Patent 1,871,027 (1928).
53. Fuller, A. D., U. S. Patent 1,942,544 (1931); U. S. Patent 2,014,798 (1932); U. S. Patent 2,173,878 (1936).
54. Kantorowicz, J., U. S. Patent 1,207,177 (1916).
55. Harvey, E. H., U. Patent 1,726,824 (1923).
56. Pierson, G. G., U. S. Patent 1,989,150 (1933).
57. Kerr, R. W., U. S. Patent 2,268,215 (1940).
58. Schopmeyer, H. H., and Felton, G. E., U. S. Patent 2,218,221 (1939).
59. Radley, J. A., Starch and Its Derivatives, Chapman and Hall, London (1940).
60. Seiberlich, J. K. H., U.S. Patent 2,347,849 (1944).
61. Vincent, G. P., U.S. Patent 2,409,085 (1946).
62. Hansen, D. W., U.S. Patent 2,432,195 (1947).

CHAPTER XII

DEXTRINIZATION

BY G. V. CAESAR

In this chapter the possible mechanisms of the degradation of starch in the dry state by the application of heat and chemicals in varying quantities are discussed. The subject matter concerns certain physical and chemical properties of commercial dextrins produced from starch by normal methods of dry conversion. The graphical data obtained for these properties form the basis of the theoretical discussion. The chemicals used in the conversions discussed are weak aqueous mineral acids which are applied by the usual method of spraying them on normally dried starch and subsequently subjecting the acidified starch to heat at different temperatures and for varying periods of time. Starch degradation products of unusual character or manufacture are not described. Discussion is confined primarily to the theoretical aspects of the commercial types of starch degradation products known to the trade as white dextrins, canary dextrins, and British guns. Additional, technical aspects of the subject will be discussed in Chapter XIII.

1. **White Dextrins.** In Fig. 87, some of the more important indices of dextrinization, *viz.* viscosity, alkali lability (1), and solubility, are plotted against time of conversion for a white corn dextrin in the low to medium range of solubility (in water at 25°C.).

The temperature range of the conversion of starch to white dextrin is relatively low, and the proportion of aqueous acids incorporated with the starch is relatively high. The effect of the aqueous acid is most strikingly revealed by the high rate of decrease in viscosity. Owing to rapid congealing, combined with the high magnitude of viscosity values, it was impracticable accurately to determine kinematic viscosity values at conversion times less than about 15 minutes. The drop in the viscosity in the early stages of conversion is obviously tremendous. Near the beginning of the conversion it can be demonstrated best, perhaps, by special methods for testing thick consistencies (2, 3). The tremendous rate of change in the early stages of conversion is clearly indicated in Fig. 87. The rate is still relatively high, even at the end of the conversion period. A somewhat similar effect is observed in the curve for percentage of solubility, although it appears that the rate of change in respect to solubility (slope of curve) is more constant. An accurate determination of solubilities below a value of 1% is difficult, but it is probable that the rate of formation of soluble substance is high at all stages of the conversion and relatively uniform, irrespective of the magnitude of the changes. Semilogarithm plots are of great assistance in showing

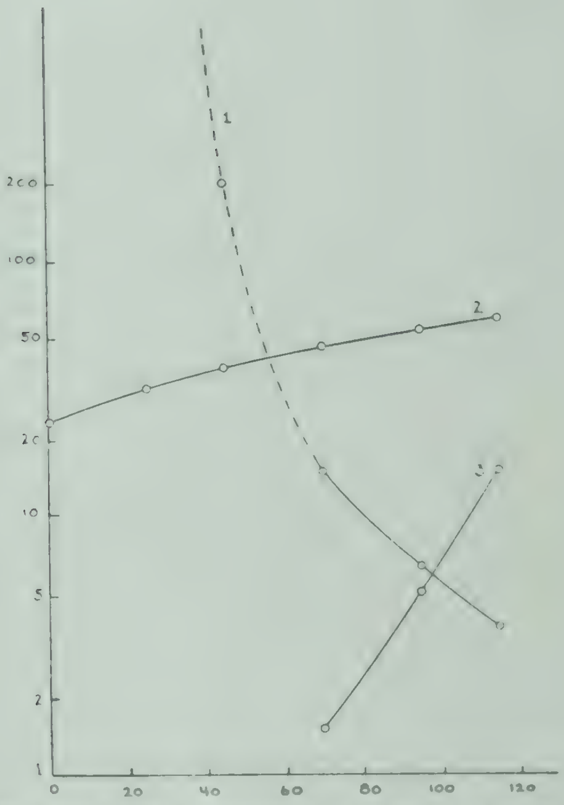


Fig. 87. Corn, white dextrin conversion. Curve 1, viscosity (centistokes); Curve 2, alkali lability, and Curve 3, solubility (per cent) plotted *versus* the time of conversion in minutes (abscissa).

these differences. The alkali lability values shown in Fig. 87 lie on a smooth curve, the slope of which slowly diminishes from a value of 23, for the unmodified corn starch, to 58 for the converted dextrin. Alkali lability is obviously a less sensitive index, in this particular conversion at least, than is either viscosity or solubility.

Let us consider the significance of these curves in respect to changes in the inner structure of the starch. Starch degradations involving hydrolytic scission of the glucoside linkages are discussed elsewhere in this volume. From a microscopic examination, the behavior in water of thin boiling starches and dextrins, particularly the white dextrins, suggests that this action, when involved in dextrinization, is highly specific. It appears that the framework of the granules has been profoundly shattered. They swell only a little, split into fragments, and show more or less solution or sloughing away of surface layers. The highly converted, soluble dextrins (white and canary) exhibit little or no preliminary swelling. The microscopic picture is highly suggestive of the snipping action of an infinitesimal pair of scissors on the molecular clusters of molecules which are packed into the granular packages. The "scissors" are $[H_3O]^+$ ions and their place of action is the 1-4 $\diagdown O \diagup$ linkage in unbranched or so called linear chains, and the 1-6 $\diagdown O \diagup$ linkage also, in in branched chains. An examination of Fisher-Hirschfelder atomic models suggests that the 1-6 links should be more susceptible to scission than the more occluded 1-4 links. In this connection, Kerr (4) remarks that "acid-converted starches produce a higher yield of butanol precipitate, which is very unstable, than is produced from original starch. This action of acid is explained as the production of new short linear chains by breaking-off the branches from the complexly constituted amylopectin types." This result is precisely what might be expected from spatial configurations, provided that the models represent with reasonable accuracy the stereoconfiguration.

Let us assume, accordingly, that the initial breakdown or hydrolysis of starch is more pronounced at 1-6 linkages than at 1-4, and that subsequent scission is more or less evenly distributed. The viscosity of aqueous dispersions of the dextrin at a given concentration and temperature (in the case of Fig. 87, 20% and 130°F.)* is then a function of chain length distribution of the snipped polymers and the structure in the liquid of the associations of these polymers probably formed hydrogen bonding.** If branched chains represent the structure of the starch component of high viscosity (4), as indeed they should, the extremely high rate of decrease in viscosity in the early stages of conversion is explained by scission of 1-6 linkages. The rate of change, later, might be expected to decrease as hydrolytic degradation shifts more and more to the narrower unbranched component. In all dextrinizations promoted by aqueous acids, the plots of the log of viscosity as a function of time are of the general form of Fig. 87. Viscosity, when it may be determined accurately,† is one of the most sensitive and significant indices of starch degradation.

The solubility curve needs little explanation beyond what has already been said. The solubility (at 25°C.) would be expected to be low until enough of the chains are sufficiently depolymerized to form a fairly stable dispersion or "solution" in water at room temperatures. It is a common and useful index of degradation and has commercial utility for various purposes.

Interpretation of data for the lability to alkali is more difficult and uncertain. Taylor's method (1) is the one heretofore employed by the author. Schoch (5) uses a modification for which he claims advantages. Taylor's technique is difficult and complicated, and reproducible results are possible only with ample experience. Exactly 50 mg. of sample are digested for 1 hour in 0.1 *N* NaOH in a boiling water bath. A color change ensues and the intensity of the brown tint is roughly proportional to the degradation suffered by the starch in dextrinizations. Excess iodine is added, and the solution is allowed to stand in the dark for a specified time. The milligrams of iodine absorbed per 100 mg. of sample are then determined. Maximum iodine absorption occurs for the white dextrins. During the preparation of the canary types, in which process heat plays an important rôle, the curve for alkali lability passes through a definite peak and then declines, often to a very considerable extent, a phenomenon also present but less marked in conversions of modified British gums. Alkali lability is probably associated with chain degradation, state of aggregation of micellar complexes, and chemical modifications, but its chemistry is still obscure. It is an interesting and useful adjunct to other quantitative indices of dextrinization.

---

*The dextrin was first cooked thoroughly at 180F° and then cooled to 130°F.

**See Chapter IX.

†Viscosity data of Fig. 87, etc., were obtained in modified Ostwald viscosimeters.

The conversion of starch to white dextrins is thus essentially a degradation or depolymerization process probably beginning with branched chains and extending to unbranched chains. The purpose of the heating at low temperature is to increase the activity of $[H_3O]^+$ ions, the primary reagent.

Fig. 88 shows the fundamental viscosity-temperature relationship of three white corn dextrins ranging in solubility from about 3% to 20% (at 25°C).‡ It is quite apparent that Curves 1 and 2 represent products of similar type or "family," but that Curve 3 is more distantly related. The rate of the change in viscosity with rising temperature increases smoothly from 70°F. (294.2°K.) to 176°F. (353.1°K.), for both Curves 1 and 2; for Curve 3 the rate of drop is more uniform, a common characteristic of homogeneous liquids. The downward trend of Curves 1 and 2 probably is indicative of a continuously increasing rate of breaking of hydrogen bonds with increasing temperature. It is suggestive of relative heterogeneity of colloidal structure. The concentration of the dextrin must be considered in making comparisons of this nature. The plot of the logarithm of viscosity *versus* $1/T$ may indicate homogeneous liquid structure at a 10% concentration but at 20% may indicate quite the reverse. True homogeneity of structure is indicated when the curve for the logarithm of viscosity *versus* $1/T$ takes the general form of Curve 3. The lower portion of the viscosity curve of Fig. 87 represents approximately the stage of conversion of the dextrins of Curves 1 and 2 of Fig. 88. The form of the viscosity-temperature curves of these dextrins is proof of their heterogeneity.

2. British Gums. These are produced essentially by a heat treatment

---

‡Data are plotted on semilogarithmic paper against the reciprocal of absolute temperature, for reasons described in Chapter IX.

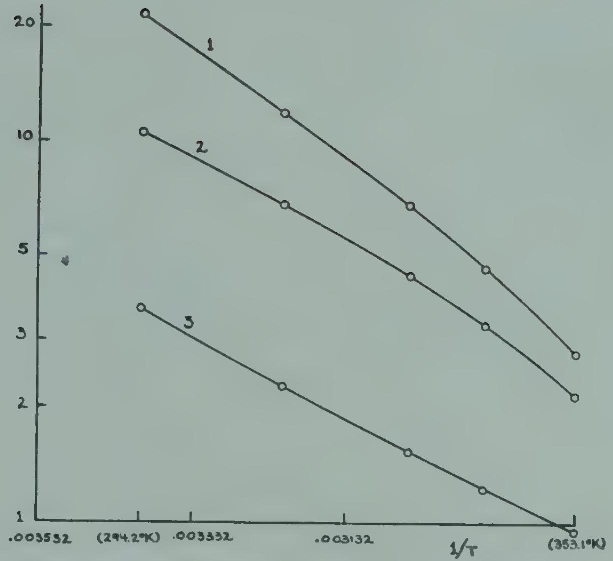


FIG. 88. Viscosity (centistokes) *versus* $1/T$ for Curve 1, white dextrin, 3% soluble; Curve 2, white dextrin, 4% soluble; Curve 3, white dextrin, 20% soluble.

of starch, although for types which might perhaps be termed "modified British" a small amount of aqueous acid may be employed to modify the more or less heavy bodied starch characteristics of ordinary British gums. Although suitable data for plotting viscosity *versus* time of conversion are not available, it may be stated that for British gums the slope of the viscosity curve, as a function of time of conversion, is much less than for conversions of white dextrin, as is also the magnitude of the change in alkali lability (6). There is some evidence that the mechanisms of heat conversion include dissociation of primary valence chains through the breaking of hydrogen bonds, dehydration to form inner ether linkages, and possible oxidation effects, particularly at high temperatures. The whole picture is extremely complex, much more so than for white dextrins, and will be further discussed under the heading, "Canary dextrins."

The viscous properties of British gums and a modified type are shown in Figs. 89 and 90. They are of interest in reflecting structural conditions. Curve 1, Fig. 89, represents a heat-converted corn starch product with about 20% of soluble material; Curve 2 is a type of modified British gum produced with the aid of acid, about 90% soluble, and of very low viscosity; Curve 3 is a canary dextrin, of still lower viscosity, almost completely soluble, also produced by heat and acid. Curve 1 shows marked structural heterogeneity which is much more pronounced than for Curve 1, Fig. 88. This reverse S form seems to be characteristic of partially disorganized starch, as discussed elsewhere.* Curve 2 likewise exhibits this peculiar form, although

---

*See Chapter IX.

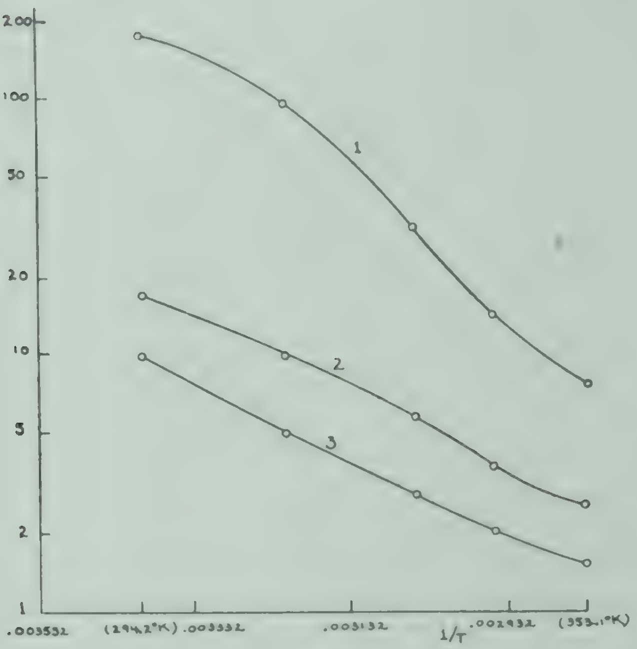


Fig. 89. Viscosity (centistokes) *versus* $1/T$ for Curve 1, British gum, heat-converted, 20%, soluble; Curve 2, British gum, heat- and acid-converted, 90% soluble; Curve 3, canary, British gum, heat- and acid-converted, 95% soluble.

the existence of the small inflections admittedly is open to question. Curve 3 has the normal form for homogeneous substances and is quite similar to Curve 3 of Fig. 88. At the 10% concentration used in the case of the latter, however, it would probably be more nearly linear.

Curve 3, Fig. 90, represents the same product as in Fig. 89. The plot of the logarithm of viscosity (at 80°F.) against percentage concentration is linear within the concentration range shown, confirming the deductions made from the plot of the logarithm of the viscosity against $1/T$. Curve 2 (Fig. 90) represents a modified British gum from corn of character intermediate between that for Curves 1 and 2 of Fig. 89. Departure from a "square law" relationship is marked, as would be anticipated from the viscosity-temperature relationship. The rate of increase in viscosity is very great. For purposes of comparison, Curve 1 (Fig. 90) shows the logarithm of viscosity *versus* concentration of an unmodified corn starch at 210°F. in a low and narrow range of concentration. This also is linear and is indicative of relative homogeneity of liquid structure *within this narrow and very low concentration range and at temperatures near boiling* where the breaking of hydrogen bonds is greatest. Reproducible data of the kinematic viscosity for corn starch are practically impossible by any technique at concentrations of more than 5% and at temperatures much lower than the boiling point.

3. Canary Dextrins. These have already been touched upon in the

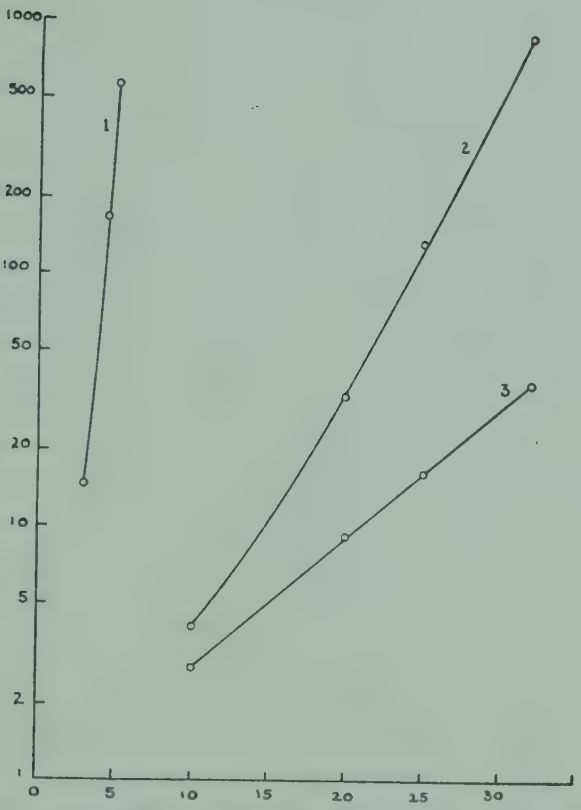


FIG. 90. Viscosity (centistokes) *versus* per cent concentration (abscissa) for Curve 1, corn starch at 210°F.; Curve 2, British gum at 80°F; Curve 3, canary, British gum at 80°F.

discussion of British gum. They are produced by the actions of heat and $[H_3O]^+$ ions and not unreasonably may be regarded as a sort of hybrid of a highly soluble white dextrin and British gum. This is indicated in Fig. 91 in which conversion time is plotted against the logarithm of the viscosity and against the lability in alkali. The general similarity of form of the viscosity curves of Figs. 87 and 91 is marked and is strongly suggestive of broad similarity of structural changes, especially in the early stages of conversion in which $[H_3O]^+$ ions play the leading rôle, probably snipping off the branched chains at the 1-6 position. In the later stages (Fig. 91) of the conversion, however, the rate of change of the viscosity diminishes noticeably and the inflection of the viscosity curve (also the inflection of the curve for solubility (6)) corresponds to the peak of the curve for alkali lability, a coincidence invariably observed in the preparation of the highly soluble canary tapioca dextrins. This coincidence seems to be true also of highly soluble canary dextrins from corn starch (6). The tapioca dextrin represented by Fig. 91 is light colored. Longer conversion would result in a darker tint, a marked drop in the alkali lability curve, and only a relatively small change

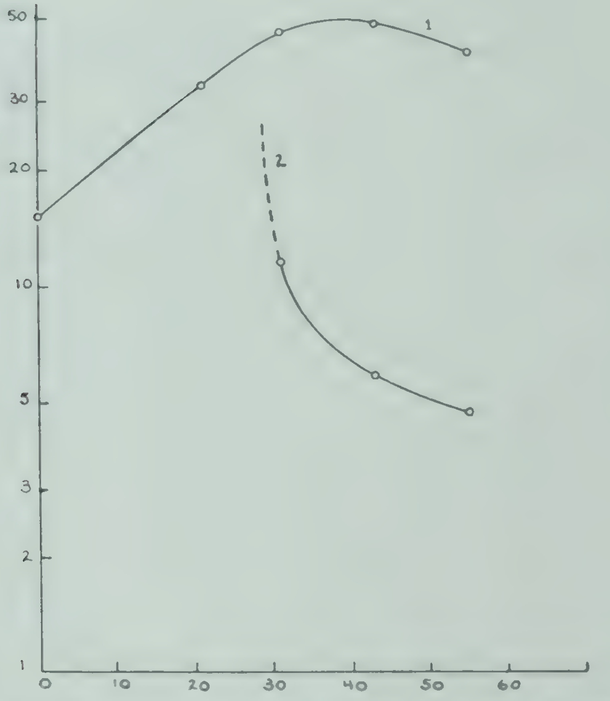


FIG. 91. Tapioca, canary dextrin conversion. Curve 1, alkali lability, and Curve 2, viscosity (centistokes) plotted *versus* time of conversion in minutes (abscissa).

in the viscosity index. The solubility would remain constant in the neighborhood of 100%.

The fluidity and homogeneity of a completely soluble (in cold water) and highly converted canary dextrin is strikingly illustrated in Fig. 92, in which the logarithm of viscosity of a tapioca dextrin solution at a concentration of 61.5% solids is plotted against $1/T$ over the temperature range of 70-176°F. (294.2-353.1°K.). The dextrin is of the type used as an envelope adhesive. The plot shows a marked approach to Newtonian viscosity* and indicates a solute structure of a very different order from that of curve 1, Fig. 89. Taylor (7) was among the first to postulate that alkali stable, inner ether anhydrides of the laevo-glucosan type were probably formed in pyrodextrins of high solubility and stable viscosity, a pioneer view which was subsequently elaborated by Brimhall (8). The latter also postulated the formation of glycide linkages by a dehydration reaction during dextrinization and this possibility was also envisaged by Taylor (7).

Recently, the author and co-workers have initiated studies on the molecular weight and intrinsic viscosity of the nitric esters formed from starch and various types of dextrin (9). The nitric esters were prepared by nitra-

---

*See Chapter IX.

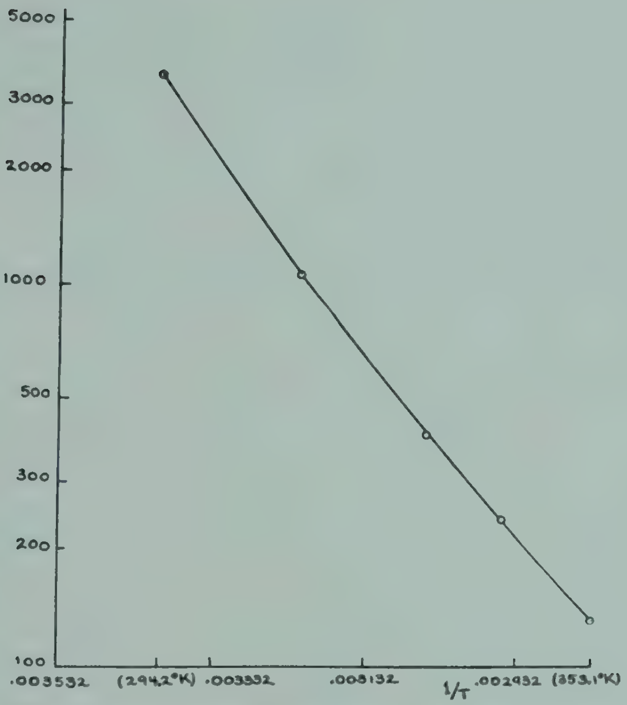


FIG. 92. Viscosity-temperature relationship for tapioca canary dextrin.

tion in chloroform solutions of N_2O_5 in the presence of NaF (10), the degradation of substrate during nitration being held to a minimum, if not—as the author believes—practically inhibited. Three series of corn starch and corn starch dextrins have been nitrated according to this technique. Series A comprised heavy and thin boiling, commercial starches prepared in aqueous inorganic acids. Series B comprised commercial corn dextrins of partial to complete solubility in cold water. Series C comprised special, highly soluble corn dextrins which had been subjected to higher conversion temperatures and longer conversion times than Series B. The mean nitrogen contents, very accurately determined, were as follows:

Dextrins	N, %	Number of samples
Series B	13.88	7
Series B	13.89	4
Series C	13.65	4

The theoretical nitrogen content for a triester having a molecular weight of the order of these dextrins is 14.14%. Series A and B approached this value, the highest values found being 14.03% in Series A, 14.01% in B, and 13.76 in C. Thus, a decided decrease in nitrogen content results when starch is subjected to high temperatures for long periods of time. These results lend considerable support to the hypothesis that inner ether anhydrides are formed during dextrinization, particularly during the latter stages of intensive heating, and it is believed that these anhydrides contain glycide cross linkages. Further studies are in progress on this subject. Periodic acid oxidation studies have thus far indicated no involvement of hydroxyls at carbons 2 or 3 in glycide anhydride formation (9), although it is possible and, for steric reasons, likely, that 6-6, glycide cross-links may be formed in addition to 1,6 laevo-glucosan groups.

Based on the evidence to date, Fig. 93 represents speculatively two possible types of canary pyrodextrins. In I, a 1-4, glucoside polymer is cross-linked through a 6-6, glycide inner ether anhydride, one terminal unit of which is a 1,6 laevo-glucosan group, and another terminal on a cross linked unit is a reducing group. In II, two 1-4, glucoside polymers are cross-linked through a 6-6 glycide ether linkage and both terminate in laevo-glucosan groups. This structure is non-reducing and stable to aqueous alkali, a property which becomes increasingly manifest in the conversion of starch by dry heating (11). Both in I and II the ether bridges should contribute to solubility and the cross-linking to viscosity. Note for example, curve 2, Fig. 91, in which the drop in viscosity, which is probably due to scission of 1-4, and 1-6, glucoside linkages, diminishes greatly with time. It is possible, furthermore, that the peak of the alkali lability curve, in Fig. 91, may be related to the formation of inner ether linkages.

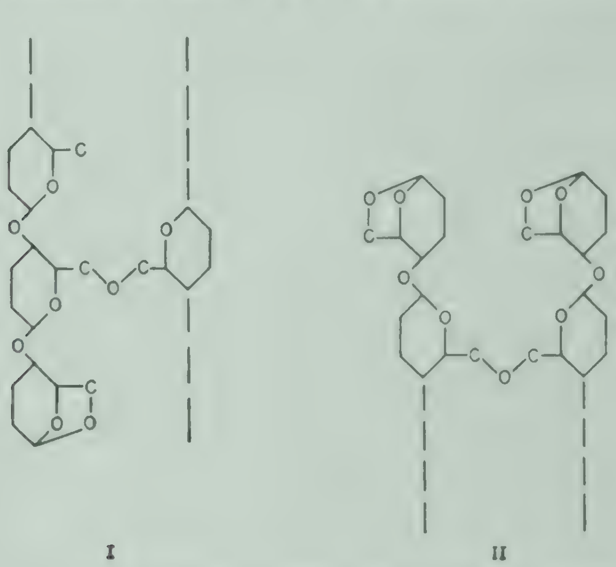


FIG. 93. Hypothetical structures for canary dextrins.

One laevo-glucosan group per polymer chain should not cause a deviation in the theoretical nitrogen content of 14.14% for a triester of nitric acid when the polysaccharide is completely nitrated. However, if more than one glucosan group per chain is formed through dehydration, then the nitrogen content for the completely nitrated polymer should be less than this value. For completely nitrated structures shown in II, Fig. 93, the nitrogen content should be 12.97%. As previously noted, the nitrogen contents of the esters in Series B approach the theoretical value for a triester and it may be assumed that their structures are broadly represented by I in Fig. 93. The nitrogen contents of the C series range from 13.76 to 13.53%. The structures present in these highly heated dextrins might be assumed to be mixtures of I and II.

Further investigations along these lines are, of course, very much in order. However, some light, it is hoped, has already been shed on a deeply fascinating subject and commercially important product.

BIBLIOGRAPHY

1. Taylor, T. C., Fletcher, H. H., and Adams, M. H., *Ind. Eng. Chem., Anal. Ed.,* **7**, 321 (1935).
2. Caesar, G. V., and Moore, E. E., *Ind. Eng. Chem.,* **27**, 1447 (1935).
3. Barham, H. N., Wagoner, J. A., and Reed, G. N., *Ind. Eng. Chem.,* **34**, 1490 (1942).
4. Kerr, R. W., *Paper Trade J.,* **115**, 30 (1942).
5. Schoch, T. J., and Jensen, C. C., *Ind. Eng. Chem., Anal. Ed.,* **12**, 531 (1940).
6. Caesar, G. V., and Cushing, M. L., *Ind. Eng. Chem.,* **31**, 921 (1939).
7. Taylor, T. C., private communications.
8. Brimhall, B., *Ind. Eng. Chem.,* **36**, 72 (1944).
9. Caesar, G. V., Gruenhut, N. S., and Cushing, M. L., *J. Am. Chem. Soc.,* **69**, 617 (1947).
10. Caesar, G. V., and Goldfrank, M, *J. Am. Chem. Soc.,* **68**, 372 (1946).
11. Caesar. G. V., and Cushing, M. L., *Ind. Eng. Chem.,* **31**, 921 (1939).

CHAPTER XIII

MANUFACTURE OF DEXTRINS

1. **Introduction.** The name dextrin has been loosely applied to a large variety of starch degradation products intermediate in molecular weight between that average for starch and the oligosaccharides. The degradation may be by acid, heat, enzymes, or other reagents, or by a combination of any of these agencies. Since these varied products are vaguely related chemically in the fact that they originated from starch and that they may be of the same order of magnitude in respect to molecular size, the name by itself, obviously, is meaningless unless prefixed by some modifying term which gives a clue as to the manner by which the product originated. The use of terms such as "torrefaction dextrin," and "β-amylase limit dextrin" has been proposed. This is better than no differentiation, but so cumbersome a system is nevertheless inadequate. Torrefaction dextrins, *e.g.*, those made by a roasting process, show a wide variation in properties and no doubt vary greatly in their chemical composition, depending on the extent of the degradation and whether a reagent is added to alter the reaction in the roasting or heating process. Acids of various types, hydrochloric, trichloroacetic, and hypochlorous, may be added. For other uses dextrins may be made by adding alkalies, such as sodium carbonate or ammonia, and organic reagents, such as urea, in the dextrinization process. When starch alone is dextrinized by heat, it has been the custom in the starch industry to call the product a "British gum." The name gum is a misnomer but the usage is so old that it probably could not be changed without confusion and inconvenience. Considerable care therefore should be exercised to avoid confusing the various types of dextrins, because of the fact that these various starch products have been grouped under one heading.

This discussion deals with the manufacture of five classes of differently constituted starch products as outlined below. These appear to be the most important industrial types of dextrins.

2. **Torrefaction Dextrins Made with No Added Catalyst (British Gums).** The simplest example of a torrefaction dextrin is the one obtained when pure starch is dried, bolted, and heated in an iron cooker with agitation. A wide variety of products results, depending on the conditions of the process. Some variable factors are (*a*) the type of starch used, (*b*) the moisture content of the starch, (*c*) the speed of heating up to the temperature of modification, (*d*) the length and manner of heating, and (*e*) the cooling and aging process. The chemistry and nature of some of these products have been discussed in other chapters. We are concerned here primarily

with manufacture and how changes in the principal variables may be expected to alter the nature of the final product. The emphasis is primarily on corn starch dextrins.

High grade tapioca and potato starches are dextrinized to more transparent products when subsequently heated with water than those obtained from corn starch. They are less short in respect to the character of the paste and when highly converted appear to "set back" less than those of corn starch, unless the latter are additionally modified. On the other hand, commercial tapioca and some grades of potato starch appear to be dextrinized with greater difficulty than corn starch. Batches of domestic sweet potato starch dextrinized by the author were found to be converted with less ease than corn starch. This difference in starches (except as noted below) is quite likely not due to the differences in the starch *per se*, but rather to impurities associated with commercial starches. Potato dextrins possess a characteristic odor and are not as desirable for some uses unless the starch be additionally modified.

For starches of very low moisture content, the degradation at first is essentially a physical one, and consists of a destruction of the orientation of the molecules in the original granule along definite lines of cleavage. Some scission in the molecular chains then follows probably at specific points that are under the greatest strain. Rearrangements follow next, quite possibly to form additional branched structures through intermolecular condensation and glucosan types of structure by intramolecular condensation.* The result is a more freely dispersing starch product with high adhesive strength for a given order of molecular magnitude. If the moisture of the starch is not reduced to very low levels and if the final temperature of dextrinization is rapidly approached in a poorly ventilated cooker, new molecular orientations result which are possibly akin to those that occur in a limited gelatinization of starch. Furthermore, for corn and similar starches at least, the acid activity of the "combined" fatty acids becomes sufficient to induce some additional hydrolytic cleavage. Hydrolytic cleavage could not be expected to be completely eliminated in this type of conversion, in any case, since the activity of the water present at these temperatures would by itself be expected to induce some scission. The result with some starches is that the dextrin does not disperse as readily in water and, at a given hot paste viscosity, tends to "set back" more on cooling. The extreme case of this type is obtained by heating starch in a pressure cooker at a high relative humidity with superheated steam. Very curious products result by this process. For example, even potato starch may be converted into a product which disperses less freely in water than native starch, and when dispersed it sets to a rigid gel according to reports of Sair.† If ventilation in the cooker is ample during the heating up period and the latter is not too rapid, then obviously the end-products will more closely approach those made in a faster dextrinization process with highly dried starch. Instead of ventilation, a partial vacuum may be applied to accomplish the same results. Most of the benefits claimed for dextrinization under a vacuum are no doubt due to a speedy and continuous removal of moisture as claimed by Krause (3).

---

*The work of Frahm (1) would suggest that condensation through 1-6 glucosidic bonds results first and that glucosans form only in the most extensive stage of the heat treatment. Brimhall (2) proposes rearrangements through 1-6 glucosidic linkages to form large branched structures.

Depending on the length of the heating period and the temperature employed, various products result. Some at one extreme differ little in physical characteristics from the starches (thin boiling). At the other extreme are those which have a very dark color and the odor of caramelized sugar. The solubility of the dextrin in cold water increases with the time of conversion. Indeed, all of these commercial dextrins, during the intermediate stages of the above conversion, are chemically very heterogeneous. Intermediate samples contain portions of cold water-soluble constituents evidently of quite low molecular weight. There are also found starch granules which have apparently been altered very little in the heating process, for all granules do not possess the same resistance to destructive agents. Small granules in a starch sample are noticeably more resistant than the largest granules. It is perhaps because commercial dextrins present such a heterogeneous composition that the subject of dextrinization by heat has attracted so little attention by those interesed in fundamental research.

Dextrinization is an art. Each manufacturing concern seems to have developed its own preferred procedures for making products to supply the trade. These processes have been developed by and the products defined according to empirical methods and procedures. The course of the dextrinization is usually followed by (a) the reduction in the viscosity of the product when a paste is formed in water, (*b*) the increase in the water-soluble content, (*c*) the changes in color, and (*d*) the change in the amount of reducing substances in the water-soluble extract. However, as might be anticipated, the product of one manufacturer is frequently not identical to that of another in its working characteristics or colloidal behavior although they correspond in respect to the values determined by the tests mentioned above. Also, one product may match another according to one or more of the above tests but may differ in regard to others.

---

†Project reports to the Corn Industries Research Foundation, the data of which have not been published.

However, in general, the process of dextrinization may be summed up as follows: After the starch is prepared, as described in Chapters II to V, it is ground in a suitable mill and passed over fine bolting silk. It then passes to equipment for redrying. A Huhn type drier is suitable for this operation. In this equipment the starch is reduced to 5% moisture content or less. It is then passed to large iron kettles provided with efficient agitation. The kettle may be loosely covered or, when vacuum or pressure is to be applied, tightly covered. Indirect heating of the kettle is preferred, owing to the fire hazard involved in heating a very dry, highly combustible substance such as starch. For this purpose the kettle may be jacketed for the circulation of steam at high pressure or of hot oil. Gas-fired kettles are still used to some extent, however. In any event, the heating should be as uniformly applied as possible and care should be taken in the design of the kettle to reduce local overheating to a minimum. For starch of low moisture content, the temperature may be raised to about 120-130°C. as fast as is consistent with the design of the cooker in respect to heating and agitation. At this temperature the heating rate should be reduced to allow the removal of the associated water, which is released at this temperature, as uniformly as possible and to avoid undue local overheating which becomes more difficult to avoid as the temperature is raised into the range necessary to produce these types of dextrins. Frequently, temperatures of dextrinization in the range of 175°C. are used, and some manufacturers prefer a shorter conversion time at temperatures in the neighborhood of 200°C. Gas-fired kettles are generally used for these short time conversions at high temperature.

The modification is allowed to proceed until the proper characteristics develop; *i.e.*, viscosity, percentage of soluble material, and color. The conversion is then stopped by cooling the dextrin. For this purpose it is usually dropped quickly to a cold water-jacketed drum in which agitation is provided to facilitate the cooling. The product is naturally very dry and hygroscopic. Some manufacturers humidify the starch at this point with moist air. Others prefer to allow the product to return to equilibrium with the atmospheric moisture naturally by an aging or storage process. The latter process has an advantage for the manufacture of certain types of dextrins in that, simultaneously with water absorption, the dextrin releases a good portion of gases that were adsorbed during dextrinization. These gases are to a large extent composed of carbon dioxide, which, if present in a "green" or unaged dextrin will be released when the dextrin is cooked with water and then tend to create an undesirable amount of foam.

The dextrin may be blended with chemicals or further treated with reagents to produce specific changes in the colloidal characteristics or working prop erties of the dextrin. For this purpose a large blender or mixer is used which, if of sufficient size, serves to homogenize the product from several dextrinizing kettles or cookers and thus insures uniformity in the product (according to the specifications mentioned). For example, borax may be blended with the dextrin to modify its flow characteristics when it is cooked into a size or adhesive or to increase its speed of drying. It has been noted in fundamental studies that the addition of borax to solutions of amylose tends to prevent gelation and spontaneous precipitation. Among other paste-modifying agents used the following may be mentioned: Henkel and Cie (4) add water-soluble phosphates; Bloede adds bisulfite (5); Wakeman (6) suggests the use of borax and a liquid pasticizer such as diethylene glycol; Bauer *et al.* (7) suggest the use of borax and a liquid plasticizer to give the gum better working properties.

Equipment suitable for the humidification and homogenizing operations have been described by several workers (8-10).

Before being finally packaged, the dextrin is bolted over fine silk, with grinding if necessary, in order to remove specks such as metallic scale from the dextrin kettle, particles of overheated starch, etc.

In all of the operations described above a very dry and dusty product is under considerable agitation. Elaborate dust-collecting systems, therefore, are used to gather and recover the starch. The atmosphere containing the starch dust is often scrubbed with water. The solids are frequently filtered out, dried, and returned to an appropriate point in the process. Momentary neglect of the operation of dust-collecting systems or of means to prevent the escape of starch dust has frequently had disastrous consequences in the destruction of life and property. Dry starch dust is a continual fire and explosion hazard. Good housekeeping and an efficient means of collecting dust is essential in any dextrin-manufacturing plant.

Variations from those outlined in the methods or equipment used in the dextrin process will be found in the patent literature. Very early, Wilson and O'Reilly (11) suggested a continuous method or process for dextrinization. Verity (12) heated starch in shallow closed containers immersed in a heated liquid in order to secure more uniform heating. Henkel and Cie (13) described a method for dextrinizing starch by pressing it between hot surfaces. Horesi (14) has patented a process wherein the dried starch is dextrinized by suspension in a very hot atmosphere. The process is a continuous one. Bode (15) described a method by which starch is completely dehydrated, suspended in a water-immiscible solvent, and mixed with hot oil in which it is dextrinized.

3. **Torrefaction Dextrins Made with Acid Catalysts.** Frequently,

a small amount of acid may be added to the starch to speed up the dextrinization. Such products may be referred to as modified British gums. On the other hand, substantial amounts of acid may be added to the starch, and the products so formed are called the white dextrins.

These products vary from the gums described in the preceding section, depending on the amount of active acid used in the process. Degradation by hydrolytic scission is undoubtedly more pronounced in this type of dextrin. To arrive at products with a given viscosity and solubility for the same heating time a lower temperature of conversion is required than when no acid is used. As a result, these acid-made dextrins are considerably lighter in color than the corresponding gums. Products of extremely low viscosity which are usable when cooked with water in a ratio of 1:1 (or even less) finally result. These products are the remoistening gums of commerce; *i.e.*, adhesives used on labels, envelopes, postage stamps, tape, and similar articles. Some of the end-products of this type of conversion are referred to in the trade as canary dextrins.

In the acid process the content of reducing sugars in the water-soluble fraction slowly increases to a maximum and then falls off in the last stages of the conversion. It is evident, therefore, that in this process a third reaction is in progress in addition to those discussed above under gums. As the sugar content increases under the influence of the acid, the latter also catalyzes a condensation or polymerization of sugars into polysaccharides which probably do not exist in the parent starch. Whether these polymerization products are of the gentiobiose type such as result when dextrose solutions are polymerized with acid* or whether they are of a more complex nature such as those obtained when dextrose is polymerized in the dry state with other catalysts† has not been ascertained. However, the extent to which this polymerization is induced depends on the activity of the acid added and on the extent of the conversion.

Hydrochloric acid is the preferred reagent for this type of dextrinization, because its acid activity is high and hence requires that little be added, because the acid is volatilized to some extent as the dextrinization proceeds and therefore neutralization of the end-product may in some cases be omitted, and because light colored products result.

Other acids may be used, however, such as lactic (17), phosphoric, and sulfuric, but more care in application is required. Otherwise, these acids tend to cause local overtreatment and to produce an increased amount of dark colored specks in the final product. The author has experimented to some extent with conversions with phosphoric acid and has found that satisfactory products which cooked to pastes of low viscosity and high mobility can be produced from corn starch by incorporating the acid with the starch in water suspension, and then filtering and drying the starch. Spraying the acid on dry starch gave poor results in the type of dextrinization equipment used. Another acid which appears to have some merits with corn starch, compared to hydrochloric acid, is trichchloroacetic acid. Bulfer and Gapen (18) suggest the use of monochloroacetic acid and chlorine. Acetic acid vapors were recommended in an early patent by O'Neill (19).

---

*The polymerization of dextrose by acids is discussed in sections treating with acid hydrolysis of starch. For further reference see the article by Berlin (16).

†It may be, however, that polymerization in the dry state also involves the formation of 1-6 glucosidic bonds. (See also Frahm (1).)

Salts such as $CaCl_2$ may be used (20). The $CaCl_2$ not only appears to catalyze the reaction of corn starch but its presence in the final product alters the colloidal properties of the cooked dextrin. If the correct amount of the salt is present, the dextrin appears to be more highly dispersed and more mobile at a given viscosity (21).

Acids or acid vapors which possess an oxidizing function may be used. Nitric acid (19), hypochlorous acid, and chlorine gas are examples of this type of reagent and, as might be anticipated, they alter the colloidal properties of the cooked dextrin. The color of dextrins treated with nitric acid is not as excellent as that produced by hydrochloric acid. The author has experimented at length with hypochlorous acid-converted dextrins in order to find a substitute method for producing the relatively expensive oxidized corn starches of commerce. The starch, in water suspension, is saturated with gaseous chlorine as described in a patent recently issued to Kerr (22). After several hours contact with the acid at room temperature, the slurry is filtered without neutralizing, and the product is dried, bolted, and dextrinized without further additions. Superior products result as compared to those of conversions with hydrochloric acid. However, it appears that a substantial proportion of the degradation is hydrolytic.

It is no small problem to promote the oxidation reaction during the dextrinization of starch. The colloidal properties of oxidized corn starches are excellent. If a similar product could be produced by dextrinization, costs might be reduced; and if the properties of oxidized corn starches could be carried into the range of the dextrins of high fluidity, these dextrins would find a ready market. It would appear that the patent of Berquist (23) might accomplish the first aim. By this method highly dried starch is treated with chlorine gas in the dextrinization procedures. The reason this method has not been more extensively used may be the difficulties arising in handling chlorine gas in the usual type of dextrinizing equipment. Fuller (24-26) partly oxidizes the starch with calcium hypochlorite and then dextrinizes it in the presence of various amounts of acid. It is claimed that the presence of $CaCl_2$ formed in the reaction also catalyzes the dextrinization. It is obvious, of course, that more or less hydrolytic scission is involved in this process.

It would seem that the second object mentioned above might be accomplished by oxidizing the starch, drying, and completing the degradation by heat alone. The very surprising result is, however, that these oxidized starches are much less susceptible to dextrinization by heat than untreated starch. It is very difficult to reduce the viscosity of an extensively oxidized corn starch to a lower value except by the use of conditions which cause a general breakdown in the starch with the production of highly colored products. Reducing the extent of the preoxidation results in a deviation from the properties of oxidized starches in proportion to the reduction in the amount of the preoxidation.

The method of manufacture of the white dextrins varies little from the methods given in the first section except for the addition of two steps (the second of which is sometimes omitted): addition of the acid catalyst and neutralization.

The usual method of incorporating an acid, such as hydrochloric, is to atomize it into a large closed wooden mixer provided with efficient agitation for the starch. An aqueous solution of 10 to 15% HCl is commonly used for atomizing. After the acid treatment the starch may be dried. It is the preferred process, however, to use very dry starch and to use acid of as high strength as is consistent with the design of the mixer for incorporating the acid uniformly with the starch. A short period of storage completes the intimate dispersion of the HCl into the starch sample.

Acid and acid catalysts may be thoroughly mixed with the starch by adding the acid to the starch in water suspension. This is followed by filtering, drying, and grinding (27-30). Perfect mixing of the starch and acid is accomplished by such a procedure, which seems to be required for acids such as phosphoric acid, when used at high concentrations. However, excessive quantities of acid must be used, since some is removed in the filtration operation and in the case of volatile acids some is lost in the drying operation.

If extensive storage of the acidified starch is employed, hydrolytic degradations will be increased in proportion to the storage time. Indeed, starches such as corn and in particular potato, will lose viscosity progressively with storage at room temperature at pH as high as 4.0.

As mentioned before, the temperature, the time of dextrinization, or both may be reduced in dextrinization with acid. Again, as in the case of gums, different combinations of time and temperature result in products with different properties. The possible variation in the acid concentration is another variable which further multiplies the number of possible products. Hence, each manufacturer of dextrin has available a long list of products produced by various combinations of variables which products can be used to supply almost any specific requirement.

The dextrins made at higher acid concentrations are frequently neutralized after conversion but those made with lesser amounts of acid are not so frequently neutralized. The result is that some dextrins of rather low pH appear on the market. This fact should be borne in mind by the user when the pH of his product or application is an important factor. He should either adjust the pH of the dextrin, during or after cooking, to the range which is consistent with providing best results in his particular application, or else he should inform the manufacturer of the pH desired in the product.

Elimination of the neutralization step has one disadvantage and one advantage in addition to the obvious advantage of a reduction in the number of steps in the manufacturing process. The disadvantage is that stability of the product, particularly the thicker dextrins, is less during a prolonged storage period before use. The advantage is that, if the product is left acid, the user completes the modification or degradation of the starch when he cooks the product. Shorter, or less drastic dextrinization procedures are therefor required, thus speeding production. If the user desires to adjust the pH of the product to suit his own wishes, this should be done after cooking. If neutralization is brought about before cooking, a slightly thicker product may result than he is able to use.

In the simplest case, neutralization may be accomplished by a dry blending with alkaline reagents. Sodium carbonate may be used. This, however, is not a preferred procedure, since the dextrin is not actually neutralized. The result is rather that its average pH is neutral. Furthermore, the tendency to produce a foamy product obviously is increased. Phosphate may also be used. Also, if alkaline modifying agents such as borax are to be blended with the product, a slight addition will of course neutralize the original acidity.

Dextrins may be more perfectly neutralized by exposure to alkaline vapors such as ammonia. This action may be accomplished by placing the dextrin in shallow containers in a large chamber into which the ammonia is introduced, or it may be accomplished in mixers provided with agitation into which ammonia gas is blown or aqueous ammonia is atomized. If the dextrin is to be artificially rehumidified, it can be accomplished during this latter type of neutralization.

Many variations of the basic process outlined above are concerned with other methods for introducing the acid catalyst. Browning and Barlow (31) and also Fielding (32) introduced acid vapors into the starch after it was placed in the dextrin kettle. In a subsequent patent Fielding (33) recommended a very dry starch for this purpose. Lenders (34) suggested adding the acid catalyst progressively to the starch as it was introduced into the dextrin kettle. Wulkan (35) would acidify a part of the starch with the required acid and add this mixture to the balance of the starch. Edson (36) found that better dextrinization could be accomplished by blending the starch and acid and heating the mixture in thin layers. Fuller (37) has described a novel process which consists in gelatinizing the starch with alkali, drying and grinding, then treating alkaline starch with chlorine and heat. Stein. Hall Mfg. Co. (38) secured beneficial results by adding a gel-inhibiting substance, such as SO_2 to starch of normal moisture content. The catalyst is then added and the mixture dextrinized. Meyer (39) has patented a process in which a volatile organic acid is used for dextrinizing and recovered by distillation and condensation. Stutzke (40) treated starch with nascent chlorine ($HCl + HNO_3$) for several hours, and then forced the starch through coils at about 1000 lbs. pressure. A double dextrinization is described in patents issued to Bruce (41, 42). In order to improve the colloidal properties of remoistening gums and other dextrins, the latter, after initial steps as outlined in the previous sections, are cooked under pressure with water until the desired properties are produced. In order to remove the sugars present in dextrins, Mathiessen and Krieger (43) suggested that a fermentation step be appended. More recently hydrogenation at high pressures and temperatures has been suggested to eliminate reducible substances (44). To produce dextrins free from lumping tendencies and other undesirable characteristics, Coppock (45) recommended additionally treating the dextrin by subjecting it to a pressure of about 1 lb. per square inch between heated plates at 70-130°F. for 3 to 15 minutes, then grinding and bolting.

4. **Torrefaction Dextrins Made with Basic Catalysts.** Basic catalysts are employed to produce gums with a minimum of hydrolytic degradation during the process. In the simplest case, a small amount of sodium carbonate is blended with the starch in the wet state before it is dried or with the dry starch. Other alkaline buffers may be used which neutraliz both the acids associated with, or liberated from, the starch and the acid produced by heat degradation of the carbohydrate. It is quite possible tha an appreciable atmospheric oxidation is also induced by heating the starch under alkaline conditions. In this manner dextrins are produced with rather high molecular weights and paste body for a given content of water-soluble material. Or stated differently, highly dispersible gums are produced which

still possess high viscosity or high "water-carrying" power. These gums are used particularly in sizing.

The usual types are produced by methods quite analogous to those already outlined in the preceding sections. If the alkaline buffer is carefully incorporated by dissolving it in a starch slurry and if the starch is carefully dried to a low moisture content before the application of dextrinizing temperatures, dextrinization may be carried into the range of highly soluble products without the production of excessively colored substances that might be expected from an alkaline roasting procedure.

An improvement in the general process and in the quality of gums produced is claimed in a patent issued to Clegg and Hilliard (46). This patent describes the use of basically reacting ammonia compounds.

A substantial improvement in technique and product is described by Caesar (47). An outline of a typical example, to illustrate the process, is given. Eighteen pounds of starch are heated to 132°C. in the dextrin kettle. At this temperature 408 g. of urea are mixed in and the temperature is raised to 193°C. over a total period of 4 hours. The urea melts at about the temperature at which it is stirred into the starch, so that the granules become coated with the melted urea. Rising temperatures then serve to decompose the urea into ammonia and products which eventually liberate more ammonia. The latter is, therefore, generated directly on the granules as they are being dextrinized and thus hydrolytic degradations, with the attendant loss in dextrin paste body, are eliminated. It is claimed that dextrins are produced which readily disperse in cold water to give liquids of high stability to "set back" and relatively high paste body.

Another variant of the general process described is disclosed by Haake and Haake (48). In this method alkaline hypochlorites are blended into the dry starch with mixing and the mixture dextrinized by heating. Products resembling the oxidized starches are said to result from such a procedure.

5. **Wet Dextrinization with Acid.** Wet dextrinization with acid is accomplished by two general processes, (*a*) one in which the starch is gelatinized and a dextrin solution results and (*b*) another in which the starch granule is not gelatinized during the process. In both processes, obviously, hydrolytic degradation predominates. The former method is discussed under acid hydrolysis and the product is referred to in the United States as a corn sirup, unmixed (C.S.U.), when corn starch is used, rather than a dextrin. When the product is to be used as a liquid dextrin, the conversion with acid is usually stopped at an earlier period in the hydrolysis than when the product is to be used essentially for its sugar content. Hence, these dextrins are referred to as unmixed corn sirup of low purity (low percentage of reducing sugars calculated as dextrose).

A variation in the method produces a dehydrated product. After the initial conversion with acid in a water medium (usually with steam pressure in a closed converter to conserve time, or acid, or both) the fluid is neutralized and passed between heated rolls, spray-dried, or flash-dried. The dehydrated product is then ground and bolted. Such a product, containing about 5% of reducing substances estimated as dextrose, is sold by one firm under the trade name of Amidex.

The second wet dextrinization method mentioned is discussed under modified starches. It is an extension of the process used for making the thin boiling starches.

The starch is stirred with water to form a slurry, and a converting acid is incorporated. Hydrochloric or nitric acid is suitable. The starch may then be partially converted by raising the temperature to between 50 and 55°C. for several hours. The converted product is filtered, without neutralization, and the wet cake is placed in converting kilns where it is further dehydrated and converted over a period of several days. The secondary conversion should not be speeded up by applying high temperatures at the start, or the starch will partially gelatinize and a gritty product will result.

The preliminary conversion should not be extended to the point at which the amount of cold water-soluble products is appreciable, since, if it is, these products are lost in the filtration operation and pass into the filtrate. In a modification of the process the initial conversion is practically eliminated and all of the conversion is performed in the kilns.

The final conversion is extended until the desired characteristics result. These vary depending on the application. The end-products are characterized by their content of water-soluble material and by their paste viscosity. Products giving a 100 g. Scott viscosity test of 50 to 75 are made with the soluble fraction as low as 10%. Others contain 30 to 40% of soluble material and give a 100 g. Scott test of 30 to 35.

The final product may be neutralized by exposure to ammonia fumes, as described in previous sections, or it may not be neutralized, depending on the use intended.

This method of conversion has been practiced for many years in the United States with but minor variations from the process originally described by Lenders (49). The product has had an ever increasing market and to-day is sold in large volume for sizing purposes, particularly for finishing textiles and for sizing paper products. Although some grades are as much as 30 to 40% water-soluble and have a reducing value as high as 19 (by alkaline ferricyanide reduction),[*] which may be compared to a value of 1.0 for starch and 269 for maltose, the product is referred to in industrial practice as a special starch. One such product is sold under the trade name Foxhead starch.

---

[*] Milliliters of 0.1 N ferricyanide per gram of carbohydrate.

A variation in the above method has been described by Stutzke (30). The starch, suspended in aqueous acid, is simultaneously converted and dehydrated by spraying the suspension into superheated steam at about 250°F.

6. **Dextrinization by Enzymes.** These conversions are described in further detail under "Modification of starch by enzymes" (Chapter XVI). The modification is more often performed by the user at the time of application. The notable exception is the adhesive manufacturer, who prepares liquid or semi-dehydrated pastes and glues, either by simply converting the starch enzymically or by mixing this product with other dextrins for particular uses. The literature contains many references to the use of various types of enzymes for the production of products referred to as "dextrins," but in some cases the products formed only vaguely resemble the carbohydrate chemists' conception of a dextrin, if indeed the concept is possible to define at all. One of these types, the so called Schardinger dextrins, will be discussed in the chapter dealing with the enzymolysis of starch (Chapter XV).

The dextrins produced by enzyme action which are of present industrial importance are made by the action of the two enzyme types, α- and β-amylase, either separately or by their combined activity in such natural mixtures as malt extracts. β-Amylase, in addition to producing a small yield of a dextrin of high molecular weight from starch, known as β-amylase limit dextrin, simultaneously produces large amounts of sugars of low molecular weight such as maltose. α-Amylase is apparently able to break into the starch molecules and set free products with longer chains than those of the sugars. Some of these may exhibit a reducing value but they are not, in the general sense, sugars. The amount of actual sugar produced is very small. Therefore, α-amylase has been termed the dextrinogenic amylase, while β-amylase is the saccharogenic amylase.

In industrial practice it is not practical to separate the two components, or types, from the mixtures in which they naturally occur. However, some sources may be decidedly richer in one type of component than another. Therefore, naturally occurring mixtures are commonly used, and conditions are employed which favor the action of the dextrinogenic or retard the activity of the saccharogenic component. β-Amylase appears to be the less sensitive to destruction by acids but is more easily inactivated by heat.

Neither enzyme appears to act rapidly on ungelatinized starch. The starch may be gelatinized by heat, which process if carried out in the presence of the added enzyme tends partly to inactivate the β constituent. The gelatinization temperature of starch is, moreover, a function of pH, and within limits, the higher the pH, the lower is the gelatinization temperature. In practice, the industrial conversion usually is carried out by adding the enzyme to a slurry of raw starch and water and heating the mixture. Then, as the starch starts to gelatinize, it is converted. It is less economical to gelatinize the starch by heat and cool to a temperature at which the enzyme activity is greatest and the rate of destruction is least, even were it otherwise desirable to do so. Conditions employed, therefore, are a balance between those required to gelatinize the starch on the one hand and those required for stability and activity of the enzyme on the other, for both α and β components are inactivated by heat even though the β component is the more sensitive.

An example is given further to illustrate this discussion. For certain sizing operations corn starch is made up as a 20% slurry with water. The pH is then adjusted to that for the optimal dextrinogenic activity of the enzyme preparation employed. For most commercial preparations this is between pH 6.0 and 6.5. Between 0.2 and 0.5% of enzyme is added, based on the dry weight of starch. The amount added will depend on the potency of the enzyme preparation and the extent of the modification desired. In some cases quantities of enzyme as high as 1% or even higher may be required The mixture is then stirred in a wooden or iron tub which should be free from fittings of enzyme-inhibiting metals, such as copper and brass. Heating is started. This may be done by the use of open steam jets. The temperature is raised to about 70°C. in about 15 minutes and then is maintained constan for 15 to 30 minutes to complete the desired conversion. The remainin active enzyme is destroyed, and the dispersion of the dextrinized starch is completed by bringing the temperature to about the boiling point. The conversion is adjusted to the proper dry solids content usually by dilutio but in rare cases by evaporation.

The writer has prepared many of these enzyme-converted dextrins in dehydrated form by roll-drying and by spray-drying the conversion liquors A small amount of borax may be included in the conversion liquors before the drying to facilitate the wetting properties of the dried dextrin, to modify its body, and to increase its drying qualities when it is to be used as an adhesive after redispersion in water.

Variations in the above process will be desirable, depending on the starch used, the sensitivity of the enzyme preparation to heat, and the pH required for peak efficiency of the enzyme. Information concerning the latter tw variables is usually supplied by the enzyme manufacturer. Lower tempera tures, or a lower pH, or a combination of lower temperatures and a lowe

16/4/92

CFTRI-MYSORE
1409
Chemistry and in

CHECKED
2008

VERIFIED
2013